

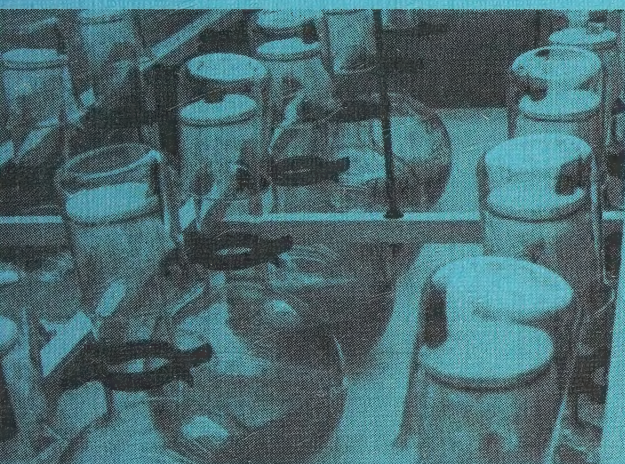


LIMNOLOGISKA INSTITUTIONEN LUNDS UNIVERSITET

ALGAL ASSAY RESEARCH IN PROGRAMS FOR EUTROPHIC LAKE MANAGEMENT

Laboratory and Field Studies

Gunilla Lindmark



INSTITUTE OF LIMNOLOGY
University of Lund

S-220 03 LUND Sweden

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LAKE MANAGEMENT

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av

Gunilla Lindmark, Vrml

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Algal Assay Research in Programs for Eutrophic Lake Management. Laboratory and field studies

Referat (sammandrag)

Algal assays were designed and applied in lake management programs. The following papers are included:

- I Influence of Interstitial Water on Growth of Selenastrum in Water from Lake Södra Bergundasjön
- II The Impact of Algae and Nutrient Composition on Sediment Exchange Dynamics
- III Effects of Environmental Stresses on the Relationship between Plectonema boryanum and Cyanophage LPP-1
- IV Phosphorus as a Growth-Controlling Factor for Phytoplankton in Lago Paranoá, Brasilia.

Results presented in a summary and above papers show how various kinds of algal assays can serve as useful tools and aid decision-making in the practical solution for eutrophication problems.

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G. Lindmark

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The dissertation includes the following papers.

- I. Influence of Interstitial Water on Growth of *Selenastrum* in Water from Lake Södra Bergundasjön.
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- IV. Phosphorus as a Growth-Controlling Factor for Phytoplankton in Lago Paranoá, Brasilia.



The papers will be referred to by the Roman numerals above.



INTRODUCTION

Nuisance algal blooms resulting from excessive nutrient input in fresh waters have become a major topic for limnological investigations. From the viewpoint of lake management and restoration, it is desirable to be able to predict consequences of changed nutrient supply for phytoplankton development.

Algal assays were designed and applied in the following lake management programs:

- 1) Lake Trummen Restoration Project
- 2) Lake Södra Bergundasjön Project for Recipient Recovery (I)
- 3) Lakes Östra Sorrödssjön and Västra Sorrödssjön, a Management Program
- 4) Lago Paranoá Restoration Project (IV)

In addition to nutrient bioassays within the above projects, algal assays have been used to investigate the impact of algae and nutrients on sediment exchange dynamics (II) and the effects of environmental conditions on relations between the blue-green alga *Plectonema boryanum* and cyanophage LPP-1 (III).

This paper presents results primarily from bioassay studies within the Lake Trummen project and the management program for Lakes Östra Sorrödssjön and Västra Sorrödssjön. Additional information on these projects is published elsewhere (Björk 1972, Lindmark 1973, Bengtsson et al. 1975, Lindmark 1975).

LAKE TRUMMEN RESTORATION PROJECT

In Lake Trummen, Vaxjö (central south Sweden), water quality



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did not improve during a decade after sewage diversion. Since the excessive blooms of blue-green algae were maintained by nutrients recycled from the sediment (Björk 1972), sediment removal as a restoration measure was undertaken in 1970 and 1971. Extensive limnological investigations were carried out before, during and after the restoration (Björk et al. 1972, Andersson et al. 1975, Bengtsson et al. 1975, Cronberg et al. 1975).

Algal assays with *Selenastrum capricornutum* were used to investigate the availability of interstitial water nutrients from different depths in the sediment and to evaluate the significance of this nutrient supply for the development of the lake.

Yield of *Selenastrum* increased by a factor of 2.5 when 10 % interstitial water from the black (cultural) sediment layer was added to lake water, while interstitial water from the underlying brown sediment layer did not stimulate algal growth (Lindmark 1973). The increase in yield was well correlated with the phosphorus contribution from the interstitial water (Lindmark 1973; Fig. 2, samples from 1970). The ratio between inorganic N and $\text{PO}_4\text{-P}$ (by weight) in the interstitial water was close to 1:1.

These results agreed well with results from studies following the restoration (Fig. 2, samples from 1972). $\text{PO}_4\text{-P}$ in the interstitial water from the newly-exposed upper brown sediment was low, and interstitial water addition to lake water did not stimulate algal growth (Fig. 2).

The change in nutrient availability in the lake water after restoration was obvious in the *Selenastrum* assays (Fig. 1). Before restoration, growth of *Selenastrum* in filtered lake water was nitrogen limited during summer. Phosphorus was in excess because sediment P was steadily released to lake water (Bengtsson et al. 1975). The inorganic N to $\text{PO}_4\text{-P}$ ratio in lake water in July, 1970, was 4:1, and this ratio was similar to inorganic N to $\text{PO}_4\text{-P}$ ratios in the sewage recipient Lake Södra Bergundasjön (I).

After restoration (1972), the significant decrease in phosphorus concentrations drastically reduced the yield of *Selenastrum* in the assays (Fig. 1). Enrichment experiments (N and/or P additions) showed that phosphorus and nitrogen together limited growth of *Selenastrum* during summer. Internal loading from the sediment was reduced, and an eventual dominance of N_2 -fixing blue-green algae due to excess P supply was avoided.

LAKE SÖDRA BERGUNDASJÖN PROJECT (I)

Investigations were initiated to evaluate the effects of sewage diversion and the role of the sediment in the recovery process (Bengtsson 1975). The growth response of *Selenastrum* in lake water and in water mixed with interstitial water was evaluated. The results were discussed in relation to phytoplankton development in the lake and the ongoing sewage diversion. Water quality was similar to that in Lake Trummen before restoration, i.e. recycling of nutrients from the sediment - P in particular - maintained excessive blooms of blue-green algae, and a shift to N_2 -fixing species had begun (Leonardson & Bengtsson 1978).

LAKES ÖSTRA SORRÖDSSJÖN AND VÄSTRA SORRÖDSSJÖN, A MANAGEMENT PROGRAM

Industrial wastewater from Perstorp AB (a plastics and chemicals industry in south Sweden) is discharged into the Ybbarpsån River which flows into Lakes Östra Sorrödssjön and Västra Sorrödssjön. A biological treatment plant was taken into operation in fall 1971 for the wastewater. Addition of phosphorus and nitrogen is required for the operation of the plant.

During 1971 and 1972 no blue-green algae were observed in the lakes, but in summer 1973 some species appeared (Berzins & Lundquist 1974). The aim of the algal bioassays was to identify the growth-controlling nutrient(s) in the lakes and to quantify the biological response to changes in growth-limiting nutrient concentrations. The results were compared with phytoplankton species composition, algal biomass and nutrient concentrations in the lakes. The results were intended to aid in designing management programs for the two lakes.

The algal bioassays were performed during summer 1974 (Lindmark 1975) using the batch culture technique according to Skulberg (1967) (see also I). *Selenastrum capricornutum* Printz, *Scenedesmus* spp. and occasionally *Ankistrodesmus* sp. and *Aphanizomenon* sp. were used as test organisms. The species of *Scenedesmus* used were cultivated from lake water collected in April, 1974, and composed a culture of 5 taxonomical units. Filtered (Whatman GF/C) lake water was enriched with various combinations of nutrients and complete nutrient medium (dilute Z8). Algal growth was measured as dry weight and by cell counts (for *Selenastrum*, Celloscope 302).

In all assays where phosphorus was added (32 $\mu\text{g P/l}$), algal yield increased (Fig. 3). The low algal growth obtained in June in Lake Västra Sörödssjön was due to the presence of only 80 $\mu\text{g/l}$ of inorganic N in the lake water. Inorganic N usually ranged between 250 and 450 $\mu\text{g N/l}$ during summer in the two lakes. Despite high concentrations of phosphorus, 70-110 $\mu\text{g total P/l}$ during summer, the *in situ* algal standing crop was moderate, i.e. 3-10 mm^3/l . Blue-green algae represented 15 % of the total algal community.

The *Scenedesmus* spp. culture usually gave a slightly higher yield than did the *Selenastrum* culture (Fig. 3). 80-90 % of total P was recovered as particulate P in the assays, and the dry matter produced per unit particulate P was within normal limits when nitrogen was in excess. Thus phosphorus was apparently the nutrient limiting algal growth in the assays. However, factors other than nutrients must also have acted to give the moderate phytoplankton biomass found in the lakes at the high total P levels. Despite this conclusion, the management program for the lakes included strict control of phosphorus in the Ybbarpsån River. The industrial wastewater treatment plant is operated to meet this requirement.

LAGO PARANOÁ RESTORATION PROJECT (IV)

In the man-made Lago Paranoá in Brasilia sewage water discharges into the lake have rapidly ruined water quality. Phosphorus was identified as the growth-controlling nutrient *in situ*. The phytoplankton in the lake - a monoculture of *Anabaenopsis raciborskii* - could assimilate nitrogen by N_2 fixation when excess

phosphorus was available in *in situ* bioassays (plastic bags). Since water renewal in the lake is rapid and no organic sediments have been deposited, recovery can be predicted if all sources of phosphorus are brought under control.

CONCLUSIONS

The results presented in this paper and in I-IV show how various kinds of algal assays can serve as useful tools and aid decision-making in the practical solution of eutrophication problems. In the use of algal assays, the most desirable model includes investigations at different levels of community complexity - bottle tests with unialgal cultures and *in situ* bag experiments as they relate to natural lake conditions.

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LAKE TRUMMEN

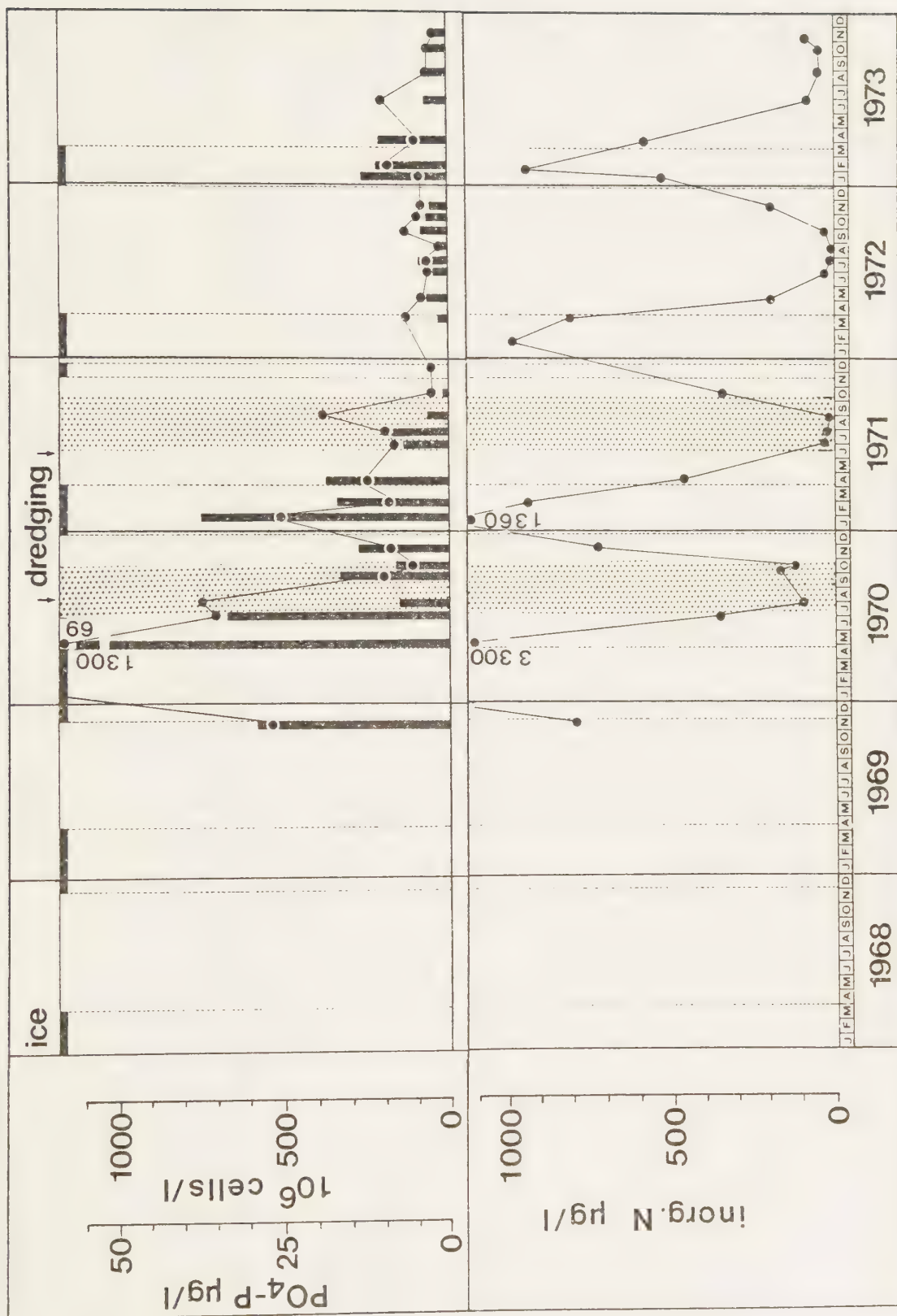


Fig. 1. Growth of *Selenastrum* in filtered lake water and concentrations of $PO_4\text{-P}$ and inorganic N following the restoration of Lake Trummen. Bars indicate cell numbers of *Selenastrum*. periods of sediment dredging and ice are indicated. (Redrawn from Bengtsson et al. 1975)

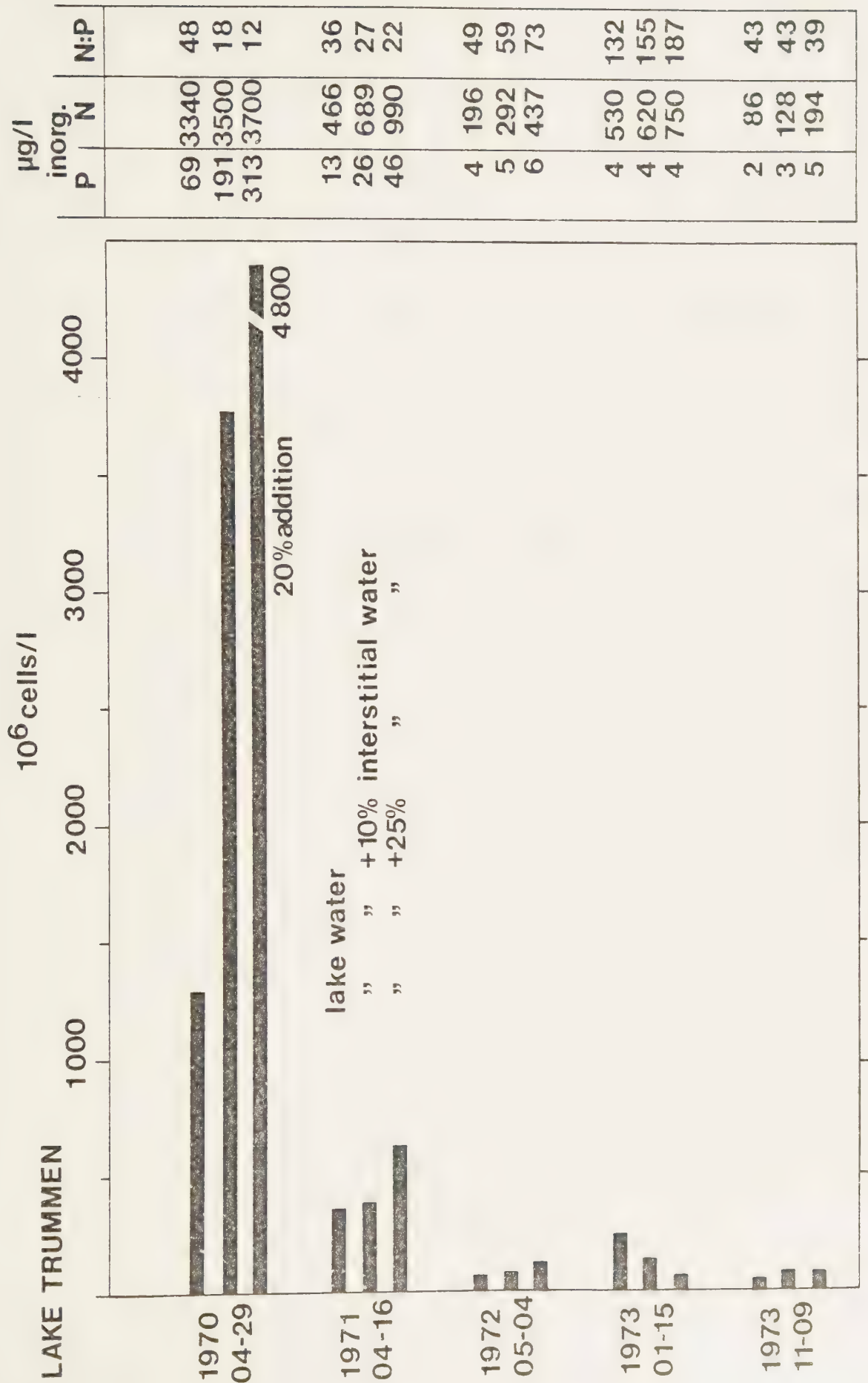


Fig. 2. Growth of *Selenastrum* in filtered lake water and in mixtures with various percentages of interstitial water from the uppermost sediment layer before (1970) and after (1972) restoration of Lake Trummen. Initial concentrations of $\text{PO}_4\text{-P}$ and inorganic N and the $\text{PO}_4\text{-P}$ to inorganic N ratio (by weight) are indicated for the assays. (From Bengtsson et al. 1975)

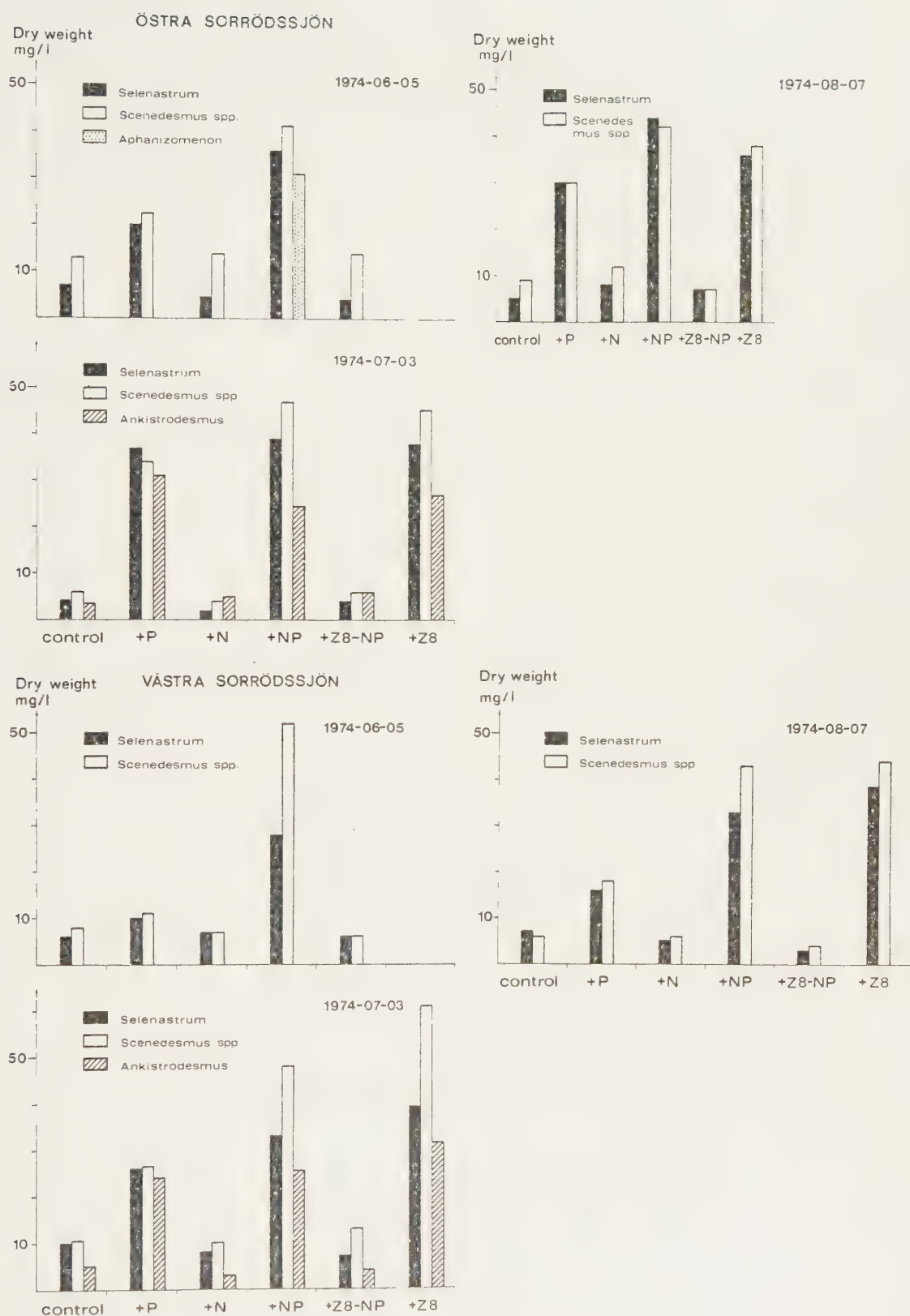


Fig. 3. Biomass of *Selenastrum capricornutum*, *Scenedesmus* spp., *Ankistrodesmus* and *Aphanizomenon* in filtered water from Lake Östra Sorrödssjön and Lake Västra Sorrödssjön enriched with various nutrient combinations. Z8 indicates a complete nutrient solution (Staub 1961).



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INFLUENCE OF INTERSTITIAL WATER ON GROWTH OF
SELENASTRUM IN WATER FROM LAKE SÖDRA
BERGUNDASJÖN

Gunilla Lindmark and Lars Bengtsson

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By Gunilla Lindmark and Lars Bengtsson

Institute of Limnology, University of Lund,
Lund, Sweden

INTRODUCTION

In evaluations of nutrient loading of lakes the sediment has to be considered as a possible internal source of nutrients for phytoplankton production. Several investigations have demonstrated how considerable amounts of nutrients can be released from sediments (cf. Kamp-Nielsen 1974, Andersen 1974, Bengtsson 1978, Ripl 1978). In some former recipients recycling of nutrients from the sediment plays a vital role in maintaining high phytoplankton biomass and seriously impairs and delays recovery after sewage diversion (Bengtsson et al. 1975, Jørgensen 1976, Ryding & Forsberg 1976, Ripl op cit.).

The relationship between nutrient concentrations in interstitial water and nutrient release from the sediment is very complex and is controlled by interacting physical, chemical and biological processes. When the sediment/water interface is undisturbed, the main mechanism for nutrient transport to lake water is molecular diffusion, and the transfer rate is dependent on concentration gradients (Duursma 1967, Stumm & Leckie 1971). In unstratified shallow lakes, however, mechanical mixing of the sediment (e.g. by wind-induced currents in the water, activities

of benthic organisms and foraging fishes) can become very significant for nutrient transport from the sediment (cf. Lee 1970). Gas release and the associated nutrient transport can be of importance in both stratified and unstratified lakes (Ohle 1965).

Algal bioassays can provide useful information to predict consequences of sediment/water nutrient exchange for phytoplankton growth. For example, laboratory experiments have shown how phosphorus in sediment and various solid substrates can be used by algae (Gahler 1969, Golterman et al. 1969, Fitzgerald 1970, Porcella et al. 1970, Ripl & Lindmark in press). The availability of sediment nitrogen for algal growth, however, seems to be more difficult to estimate, since several processes are involved and interlinked in nitrogen flux to and from the sediment (Keeney 1973, Porcella et al. 1976, Ripl & Lindmark op cit.).

The nutrients readily exchangeable are those dissolved in the interstitial water. In shallow polluted lakes the concentrations of soluble nitrogen and phosphorus are usually much higher in the interstitial water than in the overlying lake water (Gahler op cit., Bengtsson 1975) and show a certain seasonal pattern (Ripl 1979). Consequently, even minor additions of interstitial water to lake water could affect algal growth (Lindmark 1973).

Investigations were initiated in 1972 to evaluate the effects of sewage diversion and the role of sediment in the recovery process of the shallow and heavily polluted Lake Södra Bergundasjön at Växjö, central south Sweden (Table 1), (Bengtsson 1975).

The lake water is characterized by extremely high nutrient levels and excessive blooms of *Microcystis* (Table 2). In summer Secchi disc transparency never exceeds 0.5 m.

In the present investigation the growth response of *Selenastrum* in lake water and in water influenced by interstitial water nutrients - nitrogen and phosphorus in particular - was evaluated. By additional enrichment assays the growth-determining role of nitrogen and phosphorus was assessed.

The results are discussed in relation to indigenous phytoplankton development in the lake and the sewage diversion which was initiated in April, 1974.

The authors' areas of research in this paper are: Lars Bengtsson - water and sediment sampling and analysis; Gunilla Lindmark - algal bioassays and physiology.

MATERIALS AND METHODS

Samples of water (5 l), sediment and phytoplankton were collected monthly at the main sampling station (Fig. 1, max. depth 5.4 m). Water samples were taken at 0.5 m depth. Sediment was taken with a Jenkins sampler, and the phytoplankton samples (0-1 m) were collected with a plexiglass tube (length 1 m, $\varnothing$ 6 cm). The sampling was performed between 1100 and 1400 hr.

Chemical analyses and algal assays were performed on glass fiber filtered (Whatman GF/C) lake water.

The interstitial water of the sediment (0-5 cm) was obtained

by pressure (10^6 Pa) filtration (Millipore 0.45 μ m) under nitrogen.

NO_2 -N was determined according to Bendschneider & Robinson (1952), NO_3 -N as NO_2 -N after reduction by Cd-Cu, and NH_4 -N according to Chaney & Marbach (1962). Inorganic N is defined as the sum of NO_2 -N, NO_3 -N and NH_4 -N.

PO_4 -P was determined as reactive soluble phosphorus according to Murphy & Riley (1962).

Phytoplankton volumes were determined according to Willén (1974), a simplified method of the Utermöhl technique (Utermöhl 1958).

From April, 1973, to August, 1974, the algal growth response was analyzed monthly in lake water and in mixtures of 10 % interstitial water and 90 % lake water.

The assays were run as batch cultures in two-liter flat bottom round flasks with one liter test water according to Skulberg (1967). Washed and nutrient-starved cells of *Selenastrum capricornutum* Printz were added to the test water giving an initial concentration of 10^3 cells/ml. The cultures were incubated on a shaking-table (80 osc./min) and continuously exposed to 4 000 lux at a temperature of 20 ± 1 °C. Algal growth was followed by cell counts in an electronic particle counter (Celloscope 302). When the algae reached the stationary growth phase, algal yield was also determined gravimetrically (July, 1973 - June, 1974). Appropriate volumes of algal suspensions were filtered on pre-washed Whatman GF/C filters and oven dried at 105 °C.

After the first stationary phase was attained, the algal cultures were divided into two portions. $\text{NH}_4\text{-N}$ was added to one and $\text{PO}_4\text{-P}$ to the other from stock solutions of NH_4Cl and K_2HPO_4 . Concentration changes in the cultures by the N and P additions ranged from 0.6-2.7 mg N/l and 0.1-0.3 mg P/l. The resulting algal growth response was analysed as above.

RESULTS

Inorganic Nutrients

Inorganic N in the lake water showed large variations over the year (Fig. 2A). In winter and early spring ammonium dominated and reached concentrations above 2.5 mg N/l. After spring circulation ammonium was reduced, most likely due to nitrification and algal uptake, and inorganic N gradually declined to typically low summer levels. The lowest concentrations were found in August and September. However, nitrogen was never found totally depleted ($>130 \mu\text{g N/l}$, Fig. 2A).

High concentrations of $\text{PO}_4\text{-P}$ were always found (Fig. 2A). The lowest values were recorded in May (both years) after the spring flood. During summer, phosphorus, contrary to nitrogen, gradually increased (Fig. 2A).

Concentrations of inorganic N and $\text{PO}_4\text{-P}$ in interstitial water were generally about ten times higher than in lake water (Fig. 2D).

Ammonium represented $>90 \%$ of the inorganic N in interstitial

water. By the end of June, 1973, when the lake was stratified for at least four weeks, inorganic N was extremely high (27.8 mg N/l). A lowest concentration of 2.0 mg N/l was recorded in late fall. During summer, 1974, the inorganic N concentration never reached the same high levels as recorded during the previous year (Fig. 2D).

The highest concentrations of $\text{PO}_4\text{-P}$ (ca. 13 mg P/l) in the interstitial water were found in summer (July) when water temperature at the bottom was at a maximum and in February, 1974, when the lake was ice-covered. In April, 1973, the interstitial water contained only 2.2 mg $\text{PO}_4\text{-P/l}$.

Algal Growth Response

Algal growth in the filtered lake water was in most cases related to the inorganic N supply (Fig. 2A & 2B). On two occasions just after the spring flood (May, 1973 and 1974), when algal growth did not respond in proportion to available nitrogen, $\text{PO}_4\text{-P}$ concentrations were unusually low, i.e. 0.10 mg P/l and 0.20 mg P/l, respectively. As concentrations of inorganic N in the water were still considerable, high ratios of inorganic N to $\text{PO}_4\text{-P}$ (IN:IP by weight, Table 3) were obtained, indicating that P probably limited further algal growth.

In general IN:IP ratios in the lake water samples ranged between 0.16 and 7.7 with the lower values occurring at the end of the vegetation period as inorganic N concentrations steadily declined (Table 3, Fig. 2A).

In the interstitial water ratios IN:IP were even lower than in the lake water. Consequently, the addition of 10 % interstitial water to the lake water samples lowered IN:IP ratios - all were below 7.5 (Table 3) - which indicated that N rather than P would control algal growth in the assays (Chiaudani & Vigni 1974).

Algal growth in the lake water samples supplied with interstitial water was in most cases greater than in lake water alone (Fig. 2C, cf. Fig. 2B). The most significant growth stimulation occurred in summer samples and in May, 1973. In the latter case algal growth was enhanced by $\text{PO}_4\text{-P}$ in the interstitial water as later confirmed by single-element enrichment assays.

Although the N supply in the samples was increased by the addition of interstitial water, the algae did not always respond with corresponding growth (Fig. 3A). This was most obvious for samples collected in winter and early spring. It seemed that high initial concentrations of $\text{NH}_4\text{-N}$ (>2.5 mg N/l) in connection with raised pH (>10) in the cultures influenced growth conditions and possibly the nitrogen availability. When lake water $\text{NH}_4\text{-N}$ was low, interstitial water NH_4 added to the cultures gave algal growth in proportion to the N contribution (Fig. 3A).

The algal response to $\text{NH}_4\text{-N}$ added to the cultures after 14-20 days of incubation, i.e. when the algae had reached a stationary growth phase, was most obvious (Fig. 3B). On two occasions, May, 1973 and 1974, the algae were not stimulated by N addition. In all cultures that were first supplied with interstitial water, the algae responded to $\text{NH}_4\text{-N}$ addition (Fig. 3B; on two occasions

algal aggregation made cell counts impossible). Apparently all nutrients essential for algal growth were in excess of N in the filtered lake water (spring flood period excepted) and in the mixture with interstitial water.

Despite large additions of $\text{NH}_4\text{-N}$ to the cultures, IN:IP ratios remained low (two lake water samples excluded; Table 3). If $\text{NH}_4\text{-N}$ had been added to achieve IN:IP ratios above 10 the nutrient concentration would have been very high and dilution of the samples would have been necessary to get reliable algal yields.

Addition of $\text{PO}_4\text{-P}$ to the cultures stimulated algal growth only once (May, 1973). In the lake water sample from May, 1974, the algae showed the same slight response to $\text{PO}_4\text{-P}$ as to $\text{NH}_4\text{-N}$.

A typical growth response of *Selenastrum* in lake water alone and after interstitial water addition is shown in Fig. 4.

The relation between *Selenastrum* yield as cell numbers and the inorganic N supply was good ($r=0.84$, Fig. 5). A better correlation ($r=0.96$ for $n=17$) was found when only assays where inorganic N concentrations were below 2.5 mg N/l (original samples before enrichment) were considered. The relation between cell numbers and dry weight of *Selenastrum* is shown in Fig. 6. The correlation coefficient between *Selenastrum* dry weight and inorganic N was 0.95 (Fig. 7).

DISCUSSION

Algal Assay Yield in Relation to Nitrogen

The growth of *Selenastrum* in the assays was well correlated

with inorganic N, in particular when N concentrations were below ca. 2.5 mg N/l in the original (before N and P enrichment) water samples (cf. Fig. 5). Higher initial N concentrations did not give proportionally higher algal yields although N was confirmed to be the growth-controlling nutrient (cf. Fig. 5). This was true irrespective of whether the N was present in the lake water or originated from the interstitial water addition.

The lower algal yields were initially thought to be caused by carbon limitation, as pH increased to around 10 after 5-6 days incubation. However, the algal yields changed less than 10 % after the P enrichment (P-limited cultures excluded) when the cultures were incubated for another 15 days and pH had returned to around 8. Under these circumstances carbon limitation could not explain the reduced growth.

Instead, it seemed that part of the inorganic N initially present was volatilized as NH_3 at the high pH levels in the cultures ($\text{NH}_4^+ \rightleftharpoons \text{NH}_3 + \text{H}^+$; $\text{pK}=9.2$, Stumm & Morgan 1970).

When spring lake water samples from several years were examined, it was found that the algal yield coefficient for N decreased with increasing $\text{NH}_4\text{-N}$ concentration (Table 4). The maximum algal yields were similar in all samples. The impact of high $\text{NH}_4\text{-N}$ concentration was then tested by addition of $\text{NH}_4\text{-N}$ (2.5 mg N/l) at the beginning of the assay. Algal growth indicated that only a small part of the added $\text{NH}_4\text{-N}$ was assimilated, and no residual inorganic N compounds were recovered in the cultures.

The $\text{NH}_4\text{-N}$ added later (enrichment assays) was assimilated immediately, and N losses were eliminated. At this stage the cultures were denser, and the N-deficient algal cells had an increased ability for $\text{NH}_4\text{-N}$ uptake (Healey 1978). Besides, $\text{NH}_4\text{-N}$ assimilation counteracts a pH increase (Brewer & Goldman 1976).

The algal yield coefficients for N obtained in the assays were $2\,000 \cdot 10^6$ cells and 53 mg dry weight (DW) per mg N (cf. Fig. 5 and 7). The coefficients reported by Kotai et al. (1978) were $2\,500 \cdot 10^6$ cells and 62 mg DW per mg N, while Miller et al. (1978) gave a somewhat lower value, $38 (\pm 20 \%)$ mg DW/mg N. These values refer to *Selenastrum* grown in synthetic nutrient solution with NO_3 as the N source. When NH_4 was used as a N source, the *Selenastrum* yield obtained was lower than with $\text{NO}_3\text{-N}$, in particular at concentrations above 2 mg $\text{NH}_4\text{-N/l}$ (Claesson 1978).

When Healey (1978) summarized results from measurements of various degrees of nutrient deficiency in cultured algae, he could show that when the N content was lower than 2-4 % of DW, i.e. $>25\text{-}50$ mg DW/mg N, N deficiency in the algal cells was severe.

An examination of the relation between algal dry weight and cell numbers indicated that the algal biomass per cell decreased as the culture became denser, i.e. at high nutrient concentrations (cf. Fig. 6). Algal dry weight seemed, however, still proportional to nutrient concentration (cf. Fig. 7). Variations in cell size of *Selenastrum* were also pointed out in the PAAP, Final Report (Toerien et al. 1971).

Algal Assay Yield in Relation to Indigenous Phytoplankton Standing Crop

Algal assays estimate the total amount of algal cells than can grow on the nutrients present in a given sample under a fixed set of conditions (Toerien et al. 1971). However, the values usually differ from the phytoplankton biomass occurring in nature. Growth in excess of that found in the natural community indicates that the maximum possible algal concentration is rarely if ever reached in nature because of various environmental limiting factors, grazing and sedimentation of cells.

For example, in an attempt to correlate laboratory algal assay and field results, Greene et al. (1976) found a ratio of 1:1 (w:v) between *Selenastrum* dry weight (in autoclaved and filtered lake water) and the volume of the indigenous phytoplankton in a eutrophic lake.

In Lake Södra Bergundasjön the algal assays indicated that available nutrients were never totally depleted by the natural phytoplankton community. When compared with the phytoplankton standing crop in the lake, the algal volumes attained in the assays were much higher, late summer months excepted (Fig. 8).

One mg N represented a yield of approximately 110 mm^3 *Selenastrum* in the algal assays. If the particulate N fraction (total N - dissolved N) in the lake water consisted of mainly algae, the relation between particulate N and phytoplankton volume would be about 10 mm^3 algae/mg N (correlation coeff. $r=0.75$ for $n=35$ and $y\text{-intercept} = -0.6$; during 1973-1976; Bengtsson unpubl.).

In July 1974 when the maximum algal standing crop was found ($73 \text{ mm}^3/\text{l}$, Fig. 8), the phytoplankton volume per mg particulate N was slightly higher, 15 mm^3 algae/mg N. However, when the N content was determined in seston samples collected on the same occasion, nitrogen represented about 5 % of DW (Bengtsson et al. 1978). This is in good agreement with the N content in samples of *Microcystis* from heavy blooms (gathered with a plankton net; Gerloff & Skoog 1954), in lake "net plankton" from the eutrophic Lake Norrviken (Ahlgren 1977) and in various cultured algae (Healey 1978) under moderate to extreme N deficiency.

Interstitial Water Addition and Algal Response

In Lake Södra Bergundasjön nutrients in the interstitial water from the superficial 20 cm sediment layer may be available to the lake water (Bengtsson unpubl.). The volume of this interstitial water (0-20 cm) corresponds to ca. 5 % of the lake water volume. An assumed turnover time of two weeks for this interstitial water gives a minimum sediment P release of $17 \text{ mg P/m}^2 \cdot \text{day}$ (lake surface). The mean net release of phosphorus from the sediment estimated from budget calculations was $20 \text{ mg P/m}^2 \cdot \text{day}$, equivalent to an average net increase of about $8 \text{ } \mu\text{g/l} \cdot \text{day}$ in the lake water for the period May to August, 1974 (Bengtsson 1978).

With respect to phosphorus, the 10 % interstitial water added to lake water in the assays could thus appear to be too much. However, since inorganic $\text{N}:\text{PO}_4\text{-P}$ ratios (by weight) in the interstitial water were low (1-2) and nitrogen was the limiting

factor for algal growth, this addition seemed reasonable for our purpose. Since calculation of nitrogen flux between sediment and water is extremely uncertain, no attempts were made to compare the N content in the added interstitial water with the N budget (Bengtsson 1978) for the lake.

On only one occasion (May 1973) could a growth response of *Selenastrum* be traced to the phosphorus added with the interstitial water. Nitrogen in the interstitial water was well related to the additional growth, however, so ammonium in the interstitial water was an acceptable N source for *Selenastrum*.

As inorganic N concentrations in the lake water samples were extremely high from late fall to early summer, the interstitial water nitrogen added accounted for only a small part (5-25 %) of the total inorganic N. This was in contrast to summer conditions when the N added with interstitial water represented 40-91 % of the inorganic N supply. Addition of N to the lake water would thus be of greatest importance for phytoplankton development in late summer.

Consequences for Lake Management

It is evident that, at present, the influence of sediment nutrients on phytoplankton growth in Lake Södra Bergundasjön is determined by whether sediment nitrogen can be made available to the algae, since inorganic phosphorus and other essential nutrients are present in the lake water (cf. Fig. 3). The steady increase in phosphorus in the lake water during summer was partly caused by sediment release (Bengtsson 1975).

In experiments with sediment from Lake Södra Bergundasjön enclosed in dialysis bags and incubated with a *Selenastrum* culture (Ripl & Lindmark, in press), a competition for nitrogen between algae and denitrifying bacteria in the sediment was obvious. The nitrogen available was externally supplied nitrate and ammonium released from the sediment. Inorganic N was removed as rapidly in the algal-free sediment-nutrient solution system as in the system containing algae.

Even if release of sediment N can be considerable (Jacobsen & Jørgensen 1975), most of the nitrogen seems to be involved in the interlinked nitrification-denitrification processes within the superficial sediment layer and disappears from the ecosystem as molecular N. This was suggested by Messer & Brezonik (1978) after mass balance calculations and reported by Ripl & Lindmark (1978) after laboratory studies.

In laboratory microcosm experiments Porcella et al. (1976) observed that the algae (in this case mostly benthic blue-green algae) fixed free nitrogen when no external nitrogen compounds were available. They found no change in the N content of the sediment and concluded that no or insignificant use of sediment nitrogen took place. When Liao & Lean (1978 a & b) studied nitrogen transformations in water and exchange between water and sediment in large limnocorrals under various N and P loading, it was considered possible to estimate uptake and release of nitrogen in the water. However, estimations of nitrification-denitrification rates versus ammonium release from the sediment remained vague. In spite of that, they stated that

the importance of the sediment as a net source of N was insignificant in the investigated experimental ecosystems.

Although the concentration of inorganic N in the interstitial water is high in Lake Södra Bergundasjön, the release of sediment N can only have marginal effects on the N concentration in the lake water and on the quantitative development of phytoplankton. The reduced nutrient loading on the lake through sewage diversion will, however, result in a successive increase in the significance of sediment nutrients and phosphorus in particular.

Recent observations in the lake have shown that a shift in algal dominance from *Microcystis* to N_2 -fixing blue-green algal populations has begun (Leonardson & Bengtsson 1978). As this new algal community becomes established in the lake, the release of phosphorus and other nutrients from the sediment will become decisive for the development of Lake Södra Bergundasjön.

SAMMANFATTNING

Södra Bergundasjön avlastades som recipient för Växjö stad i april 1974.

Sjöns ringa djup och närsaltrika, syretärande sediment kan emellertid få stor betydelse för sjöns återhämtningsförlopp och vattenkvalitet (Tabell 2).

Målsättning i denna undersökning har varit att utvärdera effekter som inblandning av näringsrikt interstitialvatten (sedimentporvatten) kan utöva på algtillväxt i sjövattnen.

Algtester har utförts med *Selenastrum capricornutum* (Skulberg 1967). *Selenastrum* har inkuberats i dels sjövattnen, dels sjövattnen berikat med 10 % interstitialvatten och algtillväxt har bestämts som cellantal och torrsvikt. Genom separata tillsatser av fosfor och kväve har dessa ämnens algtillväxtbestämmande roll utvärderats. Resultaten har diskuterats i anslutning till fytoplanktonutvecklingen i sjön och den externa avlastningen. Undersökningen omfattar perioden april 1973 - augusti 1974.

Kraftig tillväxt av *Selenastrum* erhöles i filtrerat sjövattnen från höst till försommar. I samband med sjöns vegetationsperiod och avtagande koncentrationer av oorganiskt kväve reducerades algtillväxten märkbart, dock utan att helt upphöra (Figur 2A och 2B). Ett tydligt samband erhöles mellan oorganiskt kväve och algtillväxt (Figur 5 och 7). Endast under vårfloden i maj var fosforkoncentrationerna i sjövattnet så låga, att algtillväxten troligen var fosforbegränsad. Detta styrktes genom berikningstester. Kvoterna mellan oorganiskt kväve och fosfat i sjövattnet var i de flesta fall under 10 även efter höga (~ 2 mg/l) kvävetillsatser (Tabell 3).

Tillsats av näringsrikt interstitialvatten (Figur 2D) till sjövattnen hade störst inverkan på algtillväxten under sommarmånaderna (Figur 2B och 2C) då kvävetillskottet (>90 % $\text{NH}_4\text{-N}$) utgjorde 40-90 % av testalgernas kvävetillgång. Orsaken till att algerna inte alltid växte i proportion till kvävetillgången när $\text{NH}_4\text{-N}$ översteg ca. 2,5 mg N/l förmodades bero på kväveförluster, eftersom tillsats av ammonium efter det att algtillväxten avstannat (jfr. Figur 4) resulterade i tillväxt proportionell mot kvävetill-

satsen (Figur 3B). Förlusterna ansågs bero på att kväve försvann i form av ammoniak i samband med höga pH-värden i kulturerna (pH ca. 10 trots kontinuerlig omblandning). Endast efter vårfloden i maj 1973 stimulerades alg tillväxten av fosfortillskottet via interstitialvattnet. Genom budgetberäkningar har tidigare framgått, att sedimentet frigör fosfor under sommarmånaderna.

I genomsnitt erhöles $2\,000 \cdot 10^6$ celler och 53 mg torrvikt per mg kväve i algtesterna (Figur 5 och 7).

Resultat från dels laboratoriestudier avseende relationer mellan alger och denitrifierande bakterier (Ripl & Lindmark in press), dels kvävefixeringsstudier i sjön (Leonardson & Bengtsson 1978) tyder på, att kvävet i interstitialvattnet, trots höga halter, endast kan ha marginell betydelse för fytoplanktonutvecklingen i Södra Bergundasjön. Däremot kommer, om den påbörjade etableringen av N_2 -fixerande blågrönalger fortsätter, sedimentets betydelse som fosforkälla att öka, varvid recirkuleringen av fosfor underhåller fytoplanktonbiomassan i sjön.

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Table 1. Morphometric and hydrological data on Lake Södra Bergundasjön, Växjö, central south Sweden

Surface area	4.3 km ²	Drainage area	45.1 km ²
Volume	10.2 Mm ³	urban	ca. 30 %
Max. depth	5.4 m	lakes	13 %
Mean depth	2.4 m	Water renewal time	ca. 1 yr
Ratio organogenic sediment surface to lake surface 0.65			

Table 2. Chemical and biological characteristics of Lake Södra Bergundasjön. Minimum and maximum values (at 1 m), May - September, 1973 and 1974.

Parameter		1973	1974
Temperature	°C	11.7-23.5	11.8-18.3
Secchi disc depth	m	0.30-0.50	0.24-0.50
Turbidity	JTU	13-48	21-56
Colour	mg Pt/l	17-40	30-60
pH		7.8-9.7	8.3-9.9
Alkalinity	meq/l	0.85-1.06	0.82-1.02
Inorganic N	mg/l	0.21-2.90	0.014-1.93
Total N	mg/l	3.39-5.69	3.53-6.42
PO ₄ -P	mg/l	0.10-0.89	0.20-1.30
Total P	mg/l	0.60-1.68	0.54-1.79
Phytoplankton volume	mm ³ /l	8.3-52.3	17.3-73.1

Table 3. Ratios of inorganic N to inorganic P (by weight) in the test water before and after addition of $\text{NH}_4\text{-N}$.

Date	Lake water		Lake water + 10% interstitial water	
	original	+N	original	+N
1973-04-17	7.7	11.8	6.9	9.9
-05-17	29.4	50.0	7.5	10.8
-06-06	5.1	11.7	1.6	3.1
-06-26	2.3	5.1	2.2	3.2
-07-27	1.4	3.6	1.3	2.2
-08-22	0.16	3.1	0.88	2.1
-09-06	0.28	1.9	0.42	1.5
-10-11	1.2	3.2	1.1	2.7
-11-09	1.6	3.0	1.3	2.7
1974-02-22	3.1	5.4	1.7	2.7
-03-14	3.1	4.0	2.0	2.6
-04-16	6.5	9.5	2.5	3.6
-05-17	11.0	17.0	2.5	3.1
-06-19	2.0	4.3	1.3	2.2
-07-17	0.26	0.91	0.27	0.57

Table 4. Maximum yield of *Selenastrum*, inorganic N and P and the algal yield per unit inorganic N in lake water from Lake Södra Bergundasjön, spring samples 1971 - 1974.

Date	Algal yields 10 ⁶ cells/l	NH ₄ -N mg/l	NO ₃ +NO ₂ -N mg/l	PO ₄ -P mg/l	Algal yield/N 10 ⁶ cells/mg N
1971-04-16 ¹⁾	4900	3.7	0.43	1.00	1186
1972-05-04 ¹⁾	5000	2.3	1.06	0.56	1488
1973-04-17	5950	2.6	1.16	0.49	1582
1974-04-16	6050	2.0	0.58	0.40	2345

1) Lindmark unpubl.

LAKE SÖDRA BERGUNDASJÖN

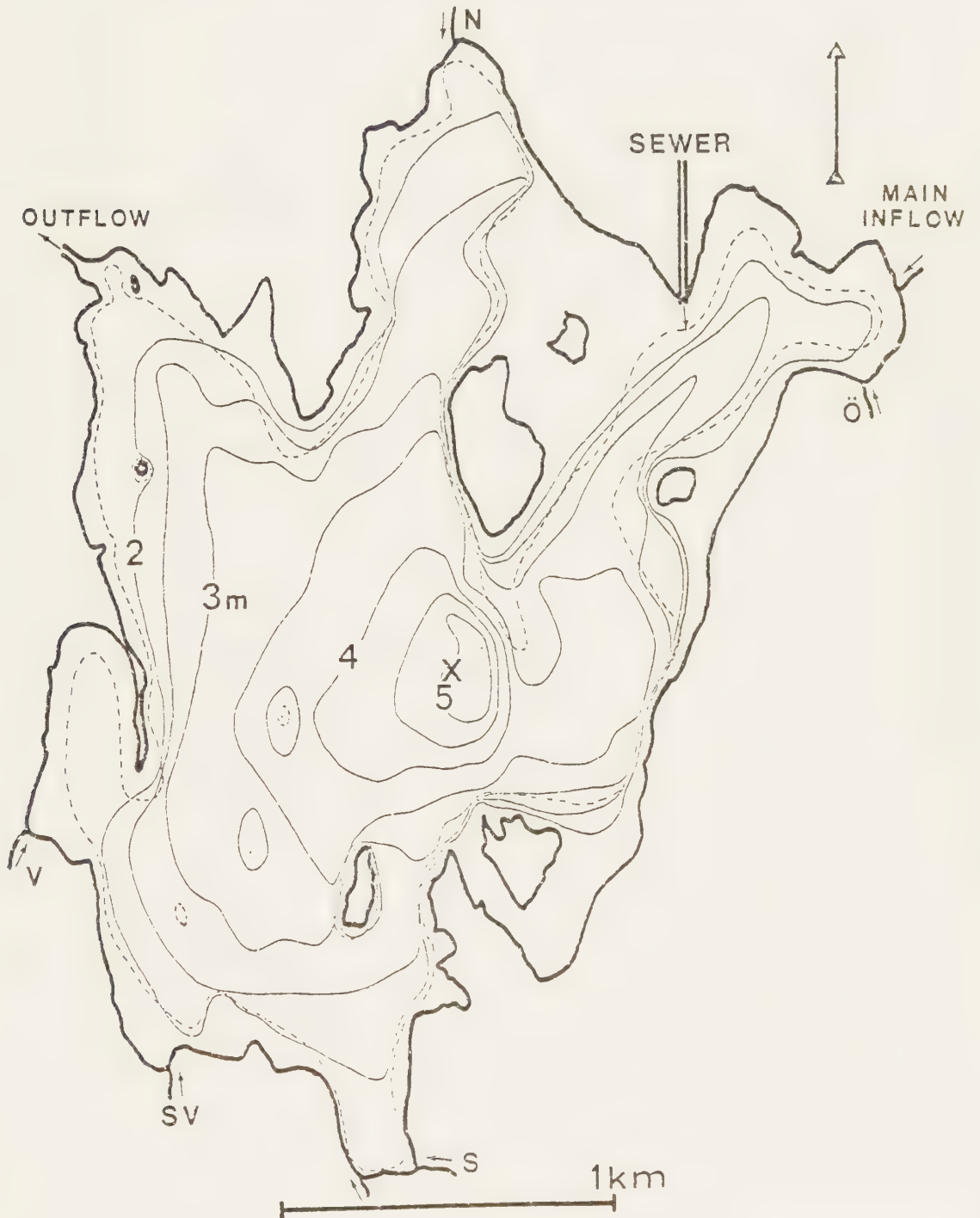


Fig. 1. Morphometric map of Lake Södra Bergundasjön.
X=sampling site. (Bengtsson 1978)

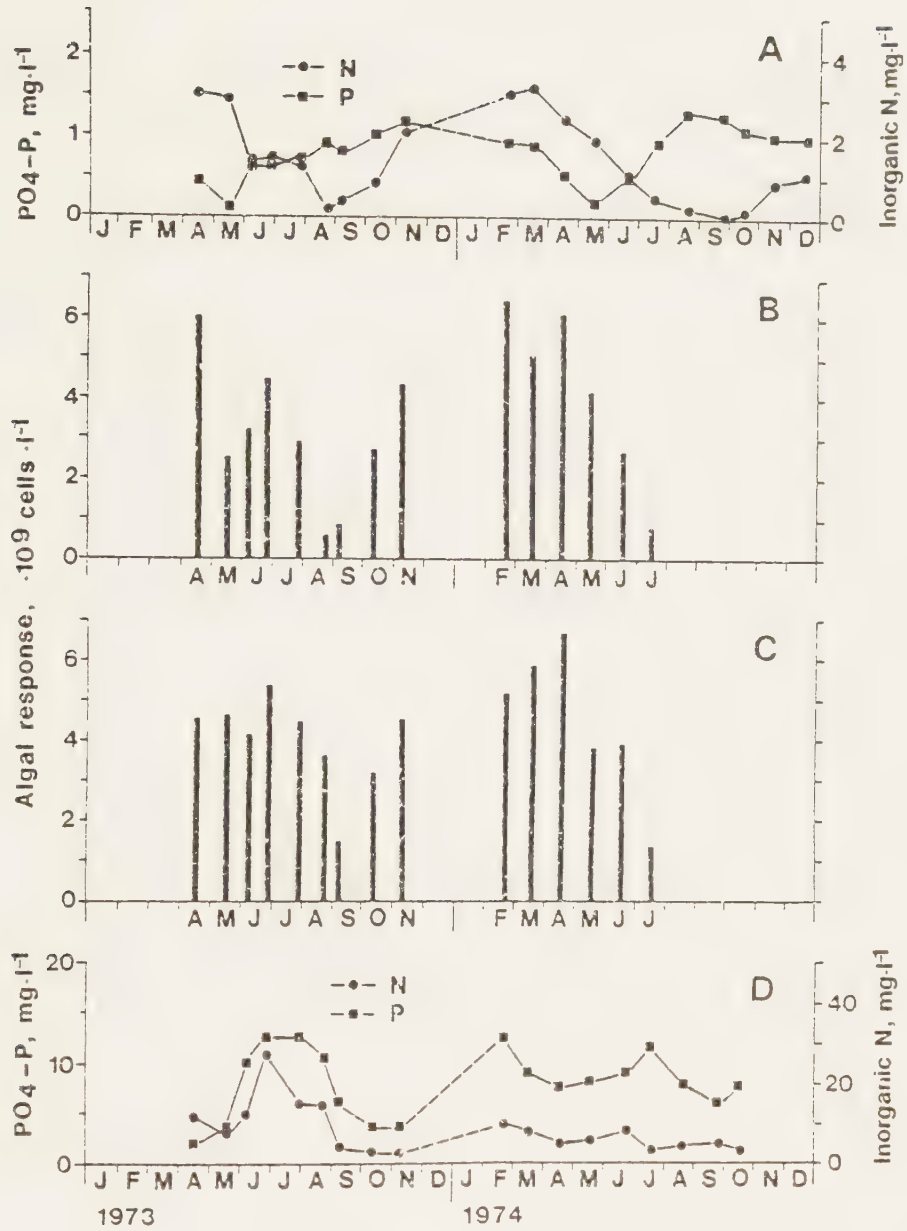


Fig 2. Lake Södra Bergundasjön, 1973 and 1974. Inorganic N and P in A) lake water and D) interstitial water, and the growth response of *Selenastrum* in B) lake water and C) lake water + 10% interstitial water.

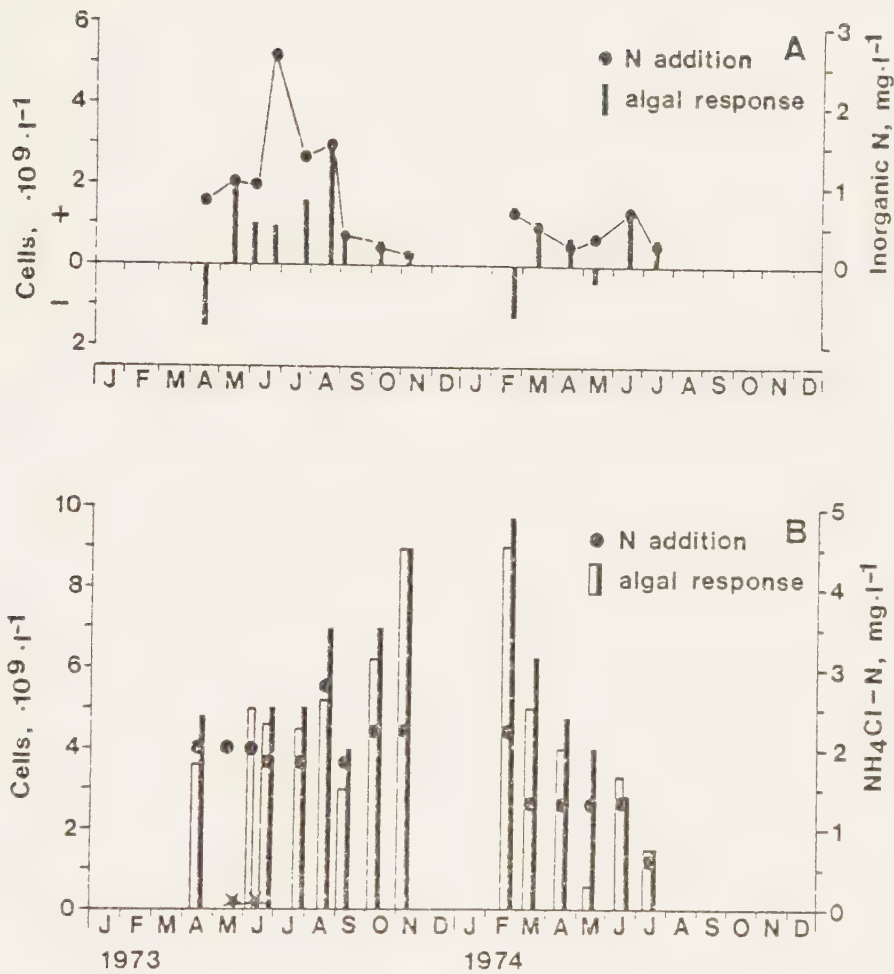


Fig 3. A. Changes in inorganic N concentration by the addition of 10% interstitial water to lake water and the growth response of *Selenastrum*.

B. Growth response of *Selenastrum* after addition of $\text{NH}_4\text{-N}$ to lake water (open bars) and to lake water + 10% interstitial water (solid bars). * indicates algal aggregation.

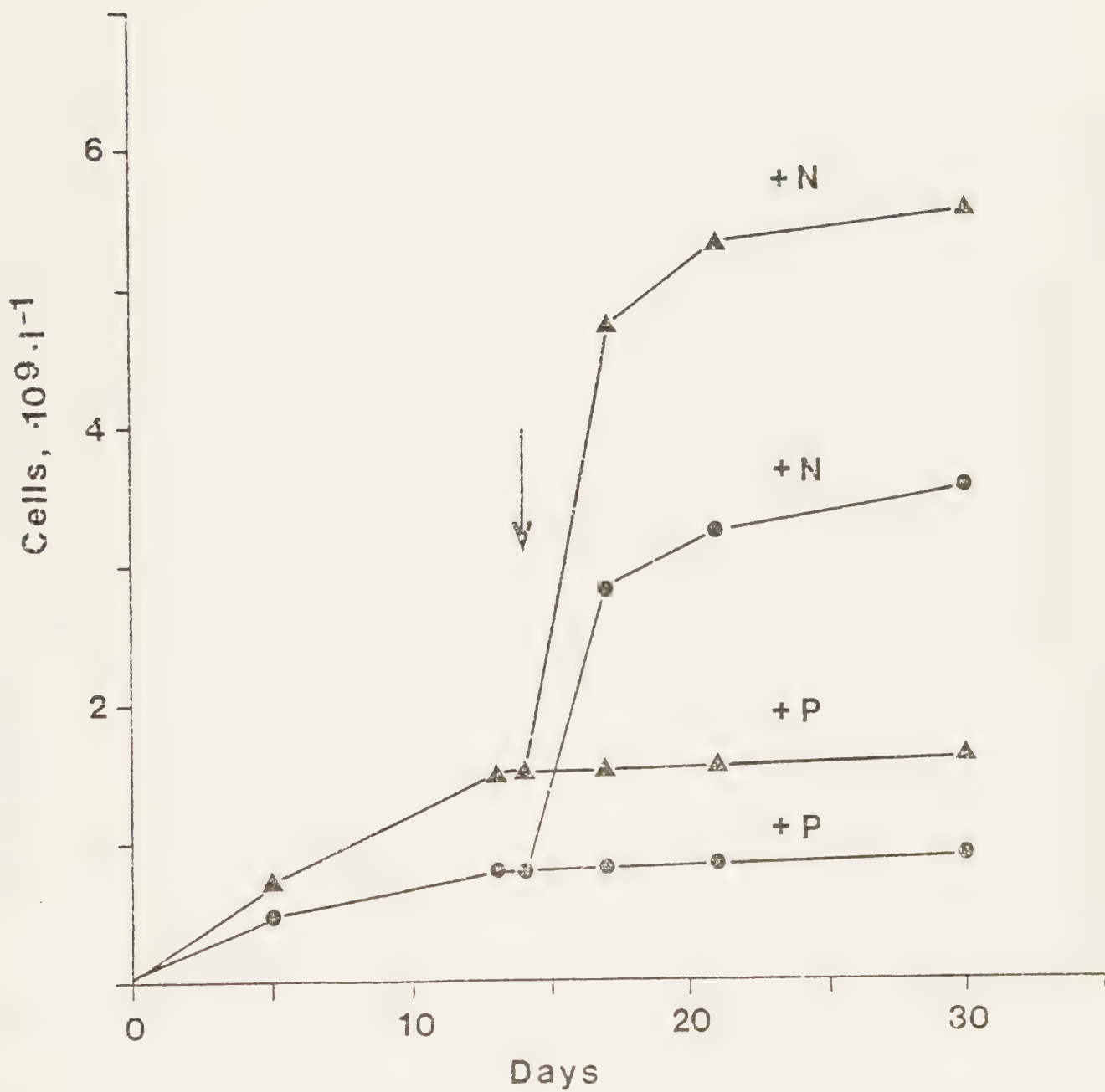


Fig 4. Growth response of *Selenastrum* in lake water (●) and lake water + 10% interstitial water (▲) September 6, 1973. Arrow indicates further addition of NH_4-N and PO_4-P .

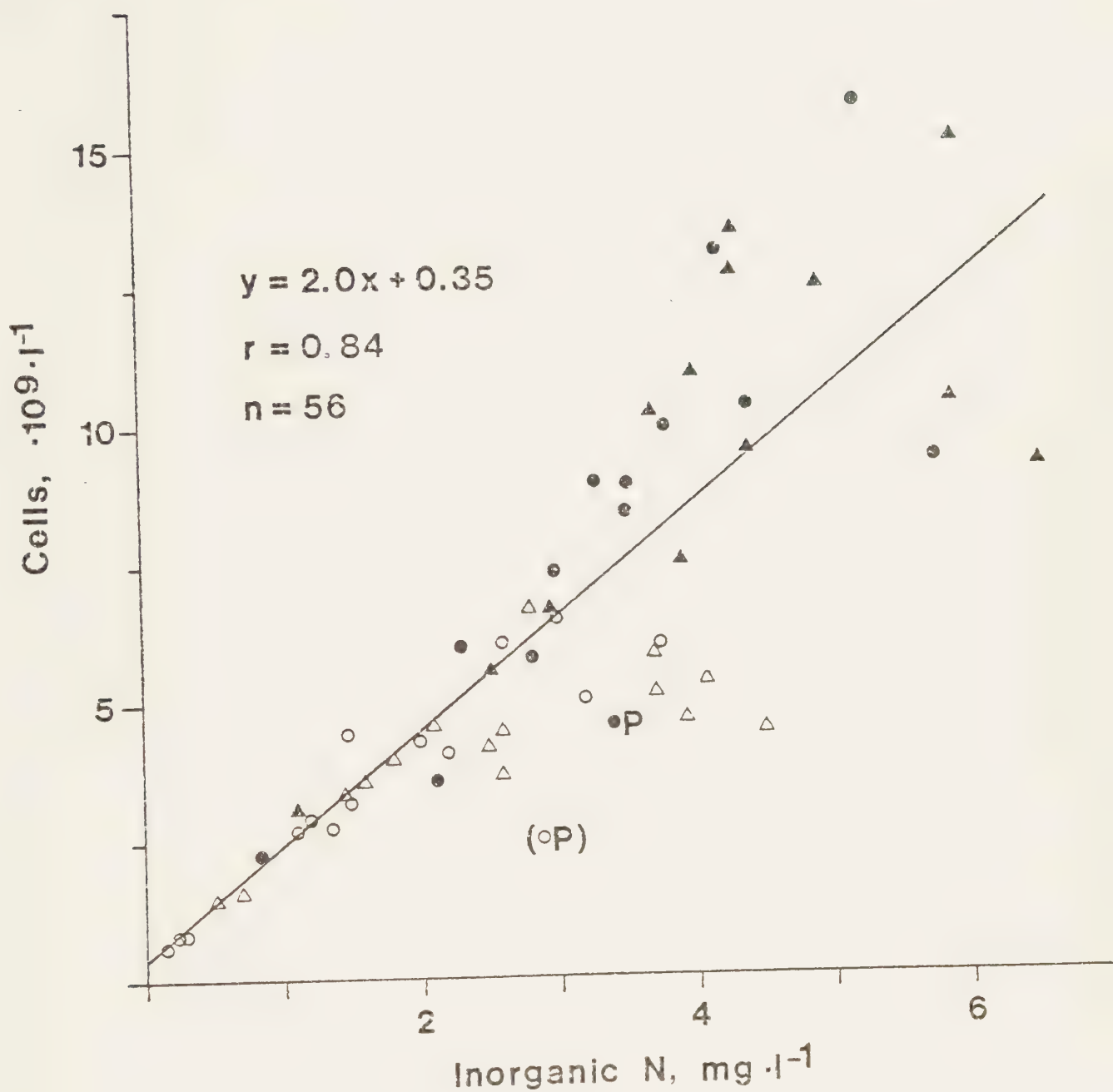


Fig 5. Growth response of *Selenastrum* as cell numbers in relation to inorganic N in lake water (O) and in lake water + 10% interstitial water (Δ). Solid symbols ($\bullet$, $\blacktriangle$) show assays after further addition of NH_4-N . The P-limited sample in brackets (May 1973) was rejected in calculation of the equation.

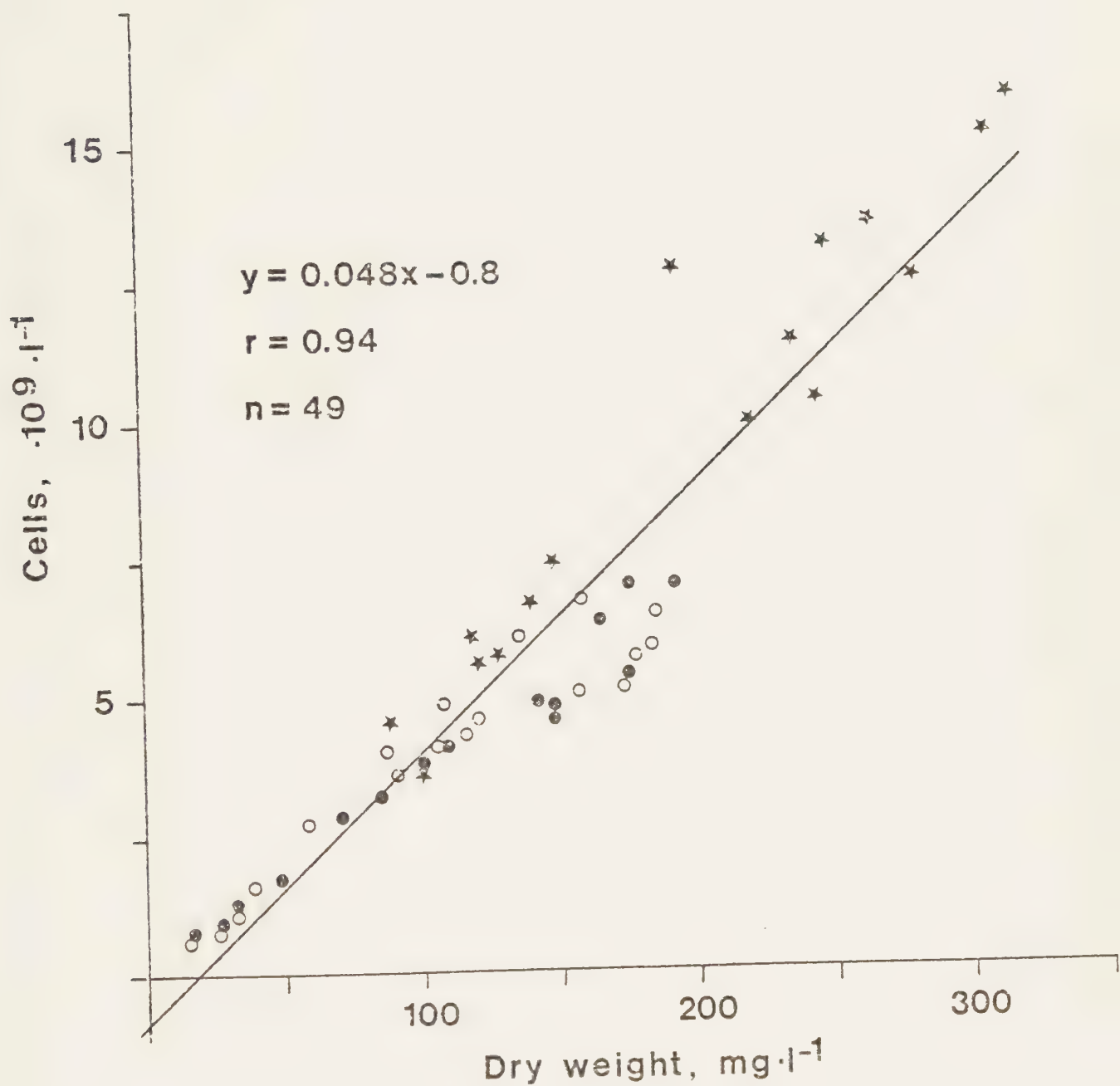


Fig 6. Relationship between cell numbers and dry weight of *Selenastrum* in lake water and in lake water plus interstitial water (O), and after addition of NH_4-N (★) and PO_4-P (●).

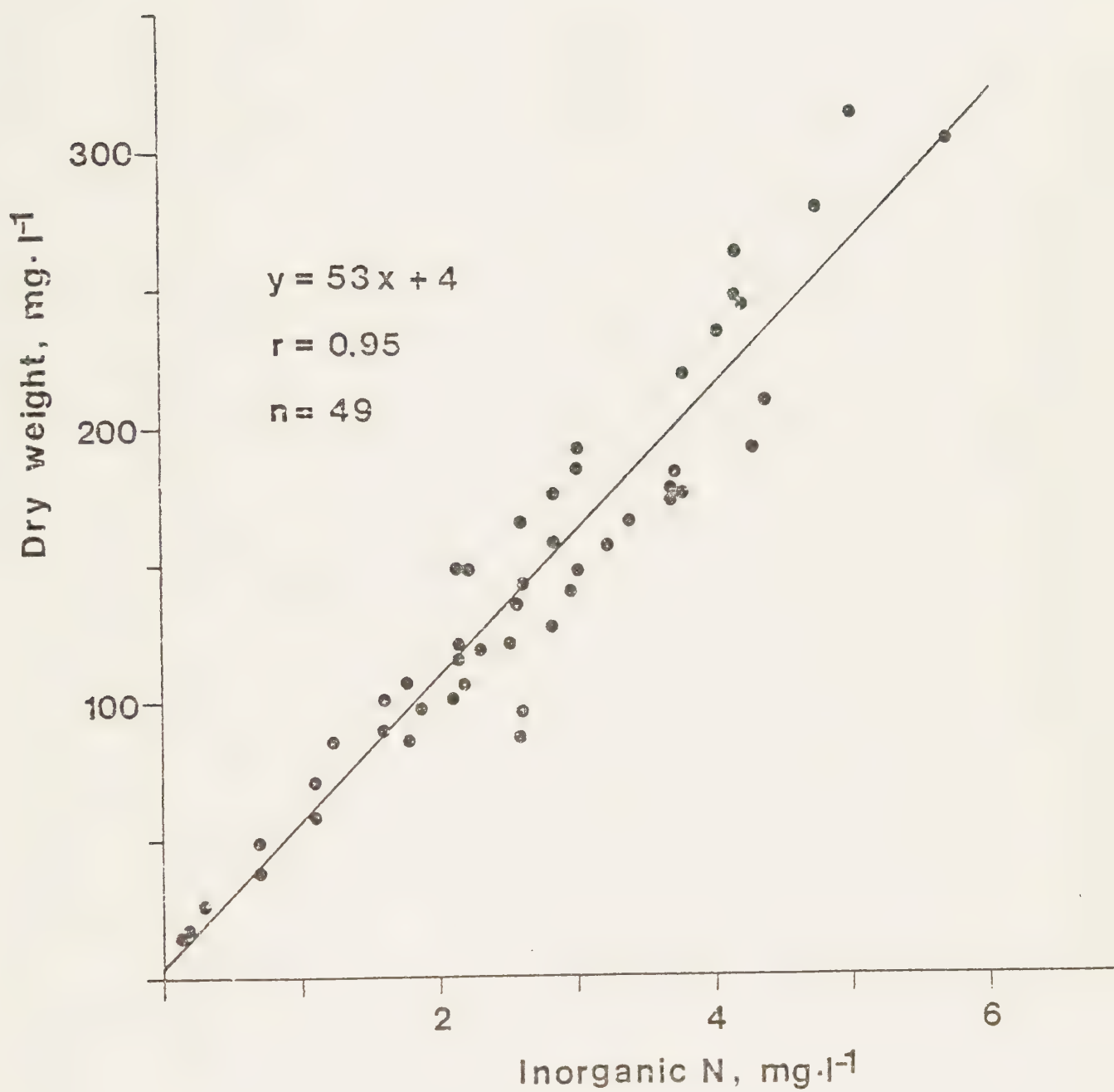


Fig 7. Growth response of *Selenastrum* as dry weight in relation to inorganic N. (Data from July, 1973 - June, 1974)

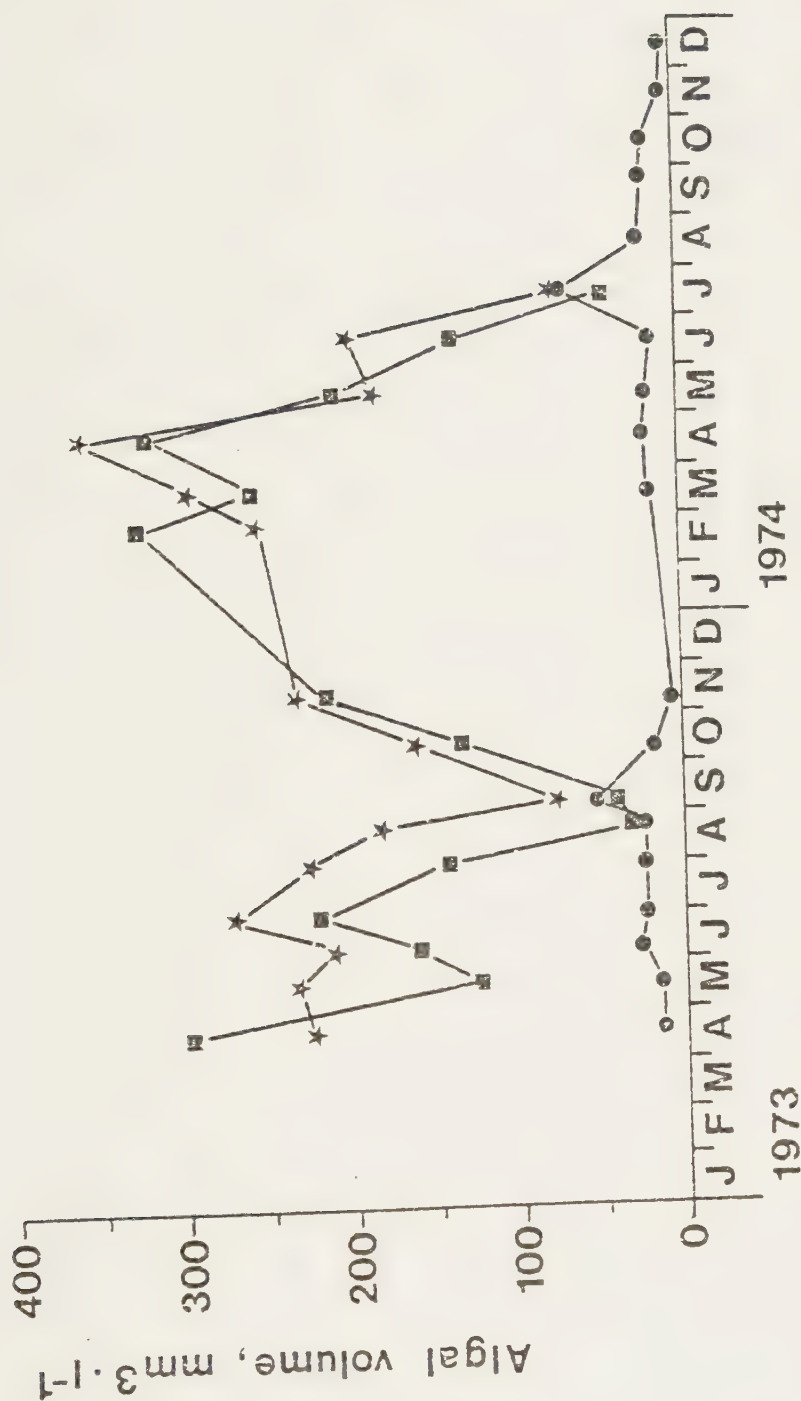


Fig 8. Indigenous phytoplankton volume (—●—) and algal assay volume for *Selenastrum* in lake water (---■---) and in lake water + 10% interstitial water (—★—) in Lake Södra Bergundasjön 1973 and 1974. (*Selenastrum* volumes were calculated using the relation between cell numbers and dry weight shown in Fig. 6 and the conversion factors $55 \mu\text{m}^3$ and $0.025 \cdot 10^{-6}$ mg DW per cell reported by Kotai et al. (1978)).



LIMNOLOGISKA INSTITUTIONEN LUNDS UNIVERSITET

THE IMPACT OF ALGAE AND NUTRIENT COMPOSITION ON SEDIMENT EXCHANGE DYNAMICS

Wilhelm Ripl and Gunilla Lindmark

INSTITUTE OF LIMNOLOGY
University of Lund

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Abstract

Laboratory systems containing a dilute nutrient solution, sediment from different lakes enclosed in dialysis bags and various concentrations of sodium bicarbonate were incubated with and without algae (*Selenastrum capricornutum*). Mutual interactions of the various system components were studied. Influence of the sediment on nutrient solution composition and algal growth was related to ionic and nutrient exchange processes. Nitrogen supply and metabolism were controlled by denitrification in and ammonia release from the sediments. Denitrifying bacterial populations operating in the sediment competed with algae for nitrate. Phosphorus supply was partly controlled by the sediments and partly by the abundance of algae, indicating that the algal populations affected phosphorus transfer from the sediments. Phosphorus was recovered as particulate (15-40 %) and soluble organic phosphorus in systems with algae, while only phosphate was found in algal-free systems. The size of the algal cells was increased when sediment was present. Possible mechanisms for sediment exchange dynamics in littoral zones and shallow lakes are discussed.

THE IMPACT OF ALGAE AND NUTRIENT COMPOSITION ON SEDIMENT EXCHANGE DYNAMICS

Wilhelm Ripl and Gunilla Lindmark

Institute of Limnology, University of Lund, Lund, Sweden

Introduction

The stimulation of algal growth due to nutrients released from sedimented organic material, mud and soil is well documented (Gahler 1969, Golterman et al. 1969, Fitzgerald 1970 a & b, Chion & Boyd 1974 and Porcella et al. 1976).

When water - sediment exchange studies are conducted in the laboratory, the role of algae is usually ignored. The exchange processes in such systems are mainly considered as controlled by sediment structure and activity. Sediment - water exchange processes have been studied under various oxygen and temperature conditions, partly by isotope techniques (Mortimer 1941, Rigler 1956, Hayes & Philips 1958, McPherson et al. 1958, Olsen 1958, Bengtsson & Fleischer 1971, Stumm & Leckie 1971, Kamp-Nielsen 1974 and Andersen 1975). Ionic exchange in sediments has been investigated by Ohle (1955), Williams et al. (1970) and Toth & Ott (1971). The role of colloids in exchange reactions was studied by Ohle (1937, 1963).

In nature aerobic and anaerobic processes occur, proceed simultaneously and are linked together in the sediment. Alternating light and dark conditions result in diel patterns of production

and respiration by algae and bacteria. Physical and chemical fluctuations are induced e.g. in pH, Eh, oxygen and carbon dioxide. These fluctuations may influence transfer rates of matter to and from the sediment surface (Lee 1970).

Studies in shallow lakes in Sweden revealed that total phosphorus in particular increased simultaneously with the outburst of planktonic algae in spring or early summer when phosphorus input from the surroundings was negligible (Gelin & Ripl 1975, Gelin 1975). In addition, algal abundance was strongly related to the rate of phosphorus exchange with the sediment, especially in waters with low alkalinity. Therefore we investigated the following topics in laboratory model systems:

1. The influence of various alkalinity conditions on the exchange of phosphorus between sediment and nutrient solutions.
2. The exchange of ions between various sediments and nutrient solutions.
3. The influence of an algal culture (*Selenastrum capricornutum*) on the exchange of phosphorus and nitrogen between sediment and nutrient solutions.

Time and spatial conditions are significant factors distinguishing natural ecosystems from laboratory model systems. Therefore, no quantitative statements about the conditions in the lakes investigated are derived from our laboratory model systems, although some mechanisms might be applicable.

The author's areas of research in this paper are: Wilhelm Ripl - water and sediment chemistry; Gunilla Lindmark - algal bio-

assays and physiology.

Material and methods

Sediment was taken from the following south Swedish lakes: Fiolen, Skärhultssjön, Hinnasjön, Trummen, Södra Berundasjön, Lillesjön (Småland, oligotrophic upland) and Vombsjön (Scania, eutrophic lowland). A variety of sediment types were thus included. Characteristics of bottom-near water, interstitial water and sediment together with morphometric data for these lakes are given in Table 1.

The sediment was sampled with an Ekman dredge during spring circulation 1973 and stored in closed plastic containers at 4°C until the experiments were conducted in late spring. 70 ml sediment was placed into a cellulose dialysis bag with membrane surface 150 cm² and approximate pore diameter 20 Å. A test of the membranes revealed that with respect to phosphorus, only reactive phosphate was able to pass. Bags with sediment from Lake Fiolen were subject to bacterial attack. The bacteria affected the membrane surface at discrete locations in both the inoculated (with algae) and the algal-free systems towards the end of the experiments (14th day). In some systems with sediment from the lakes Skärshultssjön and Hinnasjön, ruptures on the membrane appeared by the end of the experiments. For this reason some experiments were terminated earlier than others.

The experiments were run as batch algal assays (Skulberg 1967). The sediment bag and one liter of a 0.5 % Z8 nutrient solution

(Hughes et al. 1958 and Staub 1962) were placed in spherical culture flasks. The systems were inoculated with 10^6 cells/l of a washed and starved unialgal culture of *Selenastrum capricornutum* Printz.

The experimental design and incubation conditions are given in Table 2.

The growth response of the algae was evaluated by cell counts (Celloscope 302), and the maximum algal yield was determined gravimetrically (dried on Whatman GF/C filters at 105°C). The volume of the cells was estimated by approximating the shape as cylinders terminated by half-spheres.

The alkalinity in the nutrient solutions was controlled by adding various amounts of NaHCO_3 . Extra nitrogen and phosphorus were added as $\text{NaNO}_3\text{-N}$ and $\text{KH}_2\text{PO}_4\text{-P}$. Three consecutive experimental series were run (Table 2). The results from the preceding series were used in the final design of the next series.

Samples were taken from the flasks every second day by withdrawal of 30 ml of the culture. Transfer rates were corrected for the decrease in volume using a linear relationship as an approximation.

Chemical analyses were performed on water, sediment and interstitial water:

pH: electrometric with combined glass-calomel electrode.

Conductivity: Wheatstone bridge at 20°C ($\mu\text{S/cm}$).

Alkalinity: titration to pH 5.4, stripping CO_2 by N_2 .

Phosphate-P: according to Murphy and Riley (1962).

Total-P in water: digested to phosphate according to Menzel and Corvin (1965).

Nitrate-N: reduction to nitrite by a copperized Cd-column.

Nitrite-N: according to Bendschneider and Robinson (1952).

Ammonia-N: according to Chaney and Marbach (1962).

Kjeldahl-N: distillation of digested material into boric acid and acidimetric titration.

Metals (Ca, Mg, Na, K, Fe, Mn): by atomic absorption spectrophotometry (Perkin Elmer 303).

Chloride: electrometric with Ag-AgCl indicating electrode and titration with AgNO_3 in acid medium.

Sulphate: ionic exchange technique according to Mackereth (1955).

Total-P and metals in sediment and algal material were determined after digestion with a perchloric-nitric acid mixture (1:3).

Filtrations were performed with Whatman GF/C filters. Interstitial water was obtained by filtering sediments on Millipore (0.45 μm) membrane filters under nitrogen pressure and was analysed as above. Dry matter was determined after oven-drying at 105°C . Ash weight was determined after combustion at 550°C .

In text, tables and figures the following abbreviations for the sediment systems are used: Fiolen (F), Skärshultssjön (Sk), Hinnasjön (H), Trummen (T), Vombsjön (V), Södra Bergundasjön (SB) and Lillesjön (L). In series 1 sediment from all the above lakes was tested. In series 2 sediment from F, T, V and SB was

used. Series 3 was run with sediment from H, F, T, V and SB.

Results

In series 1 the effects of sodium-bicarbonate and various types of sediment on physical-chemical conditions in the experimental systems were determined (Fig. 1, 2 and Table 3). Among the control systems (no buffer added) only the V system, due to sediment properties, showed a permanent increase in alkalinity. The final concentration of total phosphorus could be related to the final algal yield in the buffered systems, while low pH - the V system excepted - insignificant algal growth (yield $<10^7$ cells/l) and low total phosphorus concentrations (16 - 40 $\mu\text{g P/l}$) characterized the control systems (Fig. 2, Table 4). In addition, the main increase in phosphorus occurred in the latter part of the experiment in the buffered systems (Table 4).

In series 2 no significant differences in phosphorus transfer, algal growth and ionic composition were obtained between systems incubated under constant light or alternating light and dark conditions. However, systems with algae showed a greater phosphorus outflow from the sediment than algal-free systems. The main increase in phosphorus occurred between the 10th and the 14th day of the experiment, the V system excepted (Fig. 3). In dark systems (Table 2) phosphorus release in the V and SB systems was higher than in the illuminated and continuously shaken systems (Fig. 3).

Two types of response pattern were distinguished in the systems with respect to inorganic nitrogen (Fig. 4). In algal-free systems the nitrate present initially (420 µg/l) in the nutrient solution was rapidly denitrified in the V and SB systems, while only minor losses of nitrate were recorded in the F and T systems. When algae were present in the solution the competition for nitrate between bacterial denitrification and algal uptake became evident (Fig. 4). This resulted in low final algal yields in the V and SB systems, while the nitrate was assimilated by algae in the F and T systems and correspondingly higher algal yields were obtained.

Ionic composition at the termination of the experiment was similar to that found in series 1. The amounts of ions exchanged between water and sediment were, however, less due to the lower buffer addition (Table 5). Alkalinity in the SB system approached zero after 9 days, and pH decreased to 6.0.

In series 3 the addition of phosphate to the experimental systems suppressed the outflow of phosphorus from the sediment when compared with systems without this addition (Table 6). In three of five systems a negative average transfer rate (flow from solution to sediment) was observed (Table 6). In all systems, however, phosphate addition resulted in higher algal growth rates (Table 7). Furthermore, the final algal yield in F, V and SB systems could be related to the initial nitrate supply in the nutrient solution (Table 7). The algal yield in the nutrient solution (0.5 % Z8) supplied with an extra addition

of phosphate was 830×10^6 cells/l. The high algal yield in the T system indicated assimilation of sediment nitrogen.

In systems where nitrate was added, the nitrogen disappeared within approximately one week. On the 10th day of the experiment another 3 mg of $\text{NO}_3\text{-N}$ was added. In the V and SB systems even these additions were denitrified within two more days. Rapid negative transfer of nitrate was observed (Table 6). Algal growth was stimulated only in the F and SB systems.

In all except the V systems, algal growth continued towards the end of the experiment, although with strongly reduced growth rate constants (0.15 - 0.35/day). In the V systems the sediment rapidly reduced the initial differences between control and nutrient-enriched systems.

At the end of the experiment all systems contained a large fraction of soluble organic phosphorus (Table 8). In systems with phosphate added, an increased amount of the total phosphorus was recovered in the particulate phosphorus fraction. In general, low concentrations of inorganic phosphorus were found in all experimental systems.

Biomass and volume of algae (calculated as μg dry weight/ 10^6 cells and $\text{mm}^3/10^6$ cells, respectively) in the control and nitrogen-enriched systems were twice as high as the values obtained in 5 % Z8 nutrient solution and in the systems containing added phosphorus (Table 9). The biomass produced per unit phosphorus incorporated was "normal" (related to cells grown in balanced culture medium) in control systems but high in systems

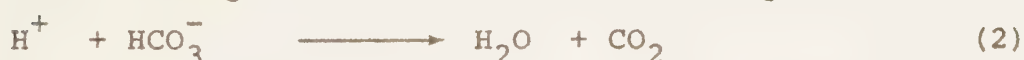
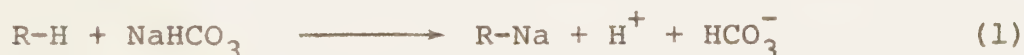
supplied with nitrogen. A luxury uptake of phosphorus was found in systems with phosphorus added.

Discussion

Different response pattern were found in the nutrient solutions depending on the three sediment types:

- A) oxidized organogenic sediments (H, F and T)
- B) reduced iron sulphide containing sediments (SB and L)
- C) calcareous sediments (V)

A) The oxidized organogenic sediments showed little outflow of ions in unbuffered solutions. When sodium bicarbonate was added, exchange occurred between acidic hydrogen ions in the sediment and sodium ions in the solution. This resulted in decreased sodium concentrations and lower alkalinity and conductivity according to the following reactions:



R = humic polyanion

Similar reactions were described by Ohle (1955) with $\text{Ca}(\text{HCO}_3)_2$ and "brown waters" rich in humic substances.

With different sodium bicarbonate concentrations in the nutrient solutions (5.5, 2.2 and 1.2 meq/l) 40 %, 44 % and 32 %, respectively, of the sodium was sorbed to the sediment (calculated for the H, F and T systems). The ratios between sorbed sodium and released CO_2 ($\Delta\text{Na} : \Delta\text{alkalinity}$) were 0.84 - 0.89 with

the highest bicarbonate addition (5.5 meq/l), 0.78 - 0.80 with 2.2 meq/l and 0.32 - 0.45 with 1.2 meq/l alkalinity. The quotients indicate a relationship between exchanged ions and the amount of sodium bicarbonate added. This may be a result of a result of a successive saturation of exchange sites in the sediments and micro-heterogeneity with respect to acidic properties.

B) The reduced iron sulphide-containing sediments lowered the alkalinity rapidly in bicarbonate-containing systems according to the oxidation reaction:



The sodium ions in these systems remained in solution, and sulphate ions were released. Similar reactions occurred in the unbuffered control systems where sedimentary calcium carbonate reacted and Ca ions were released. The amount of buffer added (NaHCO_3) affected the algae in these systems, since a depletion of the buffer substance and dropping pH values severely limited algal growth.

C) The systems containing calcareous sediment (V) sorbed some sodium in the systems with the highest amounts of bicarbonate added, but released bicarbonate ions when buffered to a lower alkalinity than that found in the lake. The sedimentary calcium carbonate was responsible for the carbonate equilibrium achieved.

These interactions between nutrient solutions and sediments influenced the inorganic carbon system and pH in the experimental

systems and thus indirectly affected algal growth and algal impact on the sediment. However, phosphorus and nitrogen exchange and metabolism in the systems were more important for the algal growth response.

The nitrogen-controlling properties of the iron sulphide-containing (SB) and the calcareous (V) sediments were obvious. The strong competitive relations between algae in solution and denitrifying bacteria in the sediment were shown by the rapid decrease of nitrate in V and SB systems incubated both with and without algae (cf. Fig. 5). Further additions of nitrate were denitrified as well, and only minor amounts of nitrate were assimilated by the algae. The depletion of nitrate by denitrification was further indicated by the increased gas content in these dialysis bags.

The high initial concentrations of phosphorus in the interstitial water seemed to favour the denitrifying bacteria in these sediments in the competition for nitrate. However, the addition of phosphate to the nutrient solution in these systems (V, SB) favoured uptake of nitrate by algae over denitrification by bacteria as indicated by high algal growth rates and final cell yields when compared to systems without phosphorus addition.

Although rich in organic matter the sediments in the F and T systems showed low denitrifying activity, and uptake of nitrate by algae dominated in both control and enriched systems.

Enhanced denitrification induced by nitrate addition to sediments from polluted lakes has recently been reported both in

laboratory studies and in whole-lake experiments (Ripl 1976, Ripl & Lundqvist 1977).

Although monitored, ammonia concentrations were measurable only at the very beginning of the experiment in systems both with and without algae. Since the ammonia concentrations were extremely low even in the algal-free systems, coupled processes between nitrifying and denitrifying bacteria were probably occurring. In systems where algae were present, a competition for ammonia between algal uptake and these processes was most probable. On several occasions ammonia from the sediments must have contributed to algal growth. In microcosm studies (Porcella et al. 1976) the algal population met its N requirement by nitrogen fixation when no external nitrogen compounds were available and showed a limited utilization of sediment nitrogen. Coupled nitrification-denitrification at the sediment water interface has been demonstrated by Keeney (1975) and Ripl & Lindmark (1978) in laboratory experiments and by Messer & Brezonik (in press) by mass balance calculations.

Phosphorus release from the sediments showed a complicated pattern. The concentration of phosphorus in the interstitial water was of minor importance for phosphorus outflow from the sediment in the illuminated systems. Sediments from the more oligotrophic lakes (F and T) with low interstitial phosphate concentrations released larger amounts of phosphorus to the solution than sediments from eutrophic lakes (V and SB). In the dark systems, which were only occasionally shaken, the outflow of phosphorus was diffusion controlled and thus related

to the interstitial phosphate concentration.

Phosphorus outflow dominated in the latter part of the experiments in the illuminated systems, but this could not be directly related to the presence of algae (cf. Fig. 3). Bacterial populations in the sediment might have affected the movement of phosphorus. The amount of phosphorus released was, however, positively correlated with algal abundance.

Only phosphate was detected in the nutrient solution of algal-free systems, while more than 50 % of total phosphorus was found as soluble organic phosphorus when algae were present (cf. Table 9). Uptake of phosphorus by algae accompanied by phosphate release from the sediment did not solely explain the accumulation of total phosphorus in the nutrient medium. Instead, complexing of phosphate by excreted organic products of algal or bacterial origin seemed to contribute to the soluble organic phosphorus pool. This is in agreement with findings of Lean (1973) and Lean & Rigler (1973) who showed a biphasic reaction of ^{32}P and transfer of phosphate to various unreactive colloidal and dissolved organic fractions when seston was present. In axenic cultures of various algal species, dissolved organic P was observed when the algae entered the stationary phase of growth (Lean & Nalewajko 1976).

When the systems were incubated with excessive amounts of phosphate, the sorption properties of some sediments became enhanced. In the V and SB systems, 40 to 50 % of the initial phosphate was removed from the nutrient solution. Although the addition of

phosphate increased phosphorus uptake by algae, large amounts of the phosphate added was recovered as soluble organic phosphorus, which emphasizes complexing reactions in the solution.

The low algal growth rate constants ($0.15 - 0.35$ / day) observed at the end of the experiment indicated that mineralization probably controlled the supply of nutrients from the sediments (Lee et al. 1976). In the V systems, however, algal growth ceased and no nutrients were supplied by the sediment.

Size and P content of the algal cells were influenced by the presence of sediment in the nutrient solution, irrespective of sediment properties. The extra additions of N and P influenced cell size and P content due to the extreme N/P composition of the solution.

Limnological synthesis.

The interactions between the sediments and algal populations in the nutrient solutions can be described as follows:

1. In solutions with extra phosphate added, the indirect influence of the sediment on the algae due to competition for nitrate was minimized.
2. In solutions with extra nitrate added, algal growth was affected only slightly, and the additional nitrate was denitrified at increased rates in the sediment.
3. In systems with a balanced nutrient solution (Z8), the influence of the sediment on the algae was determined solely by sediment properties, but more phosphorus was released

from the sediment when algae were present.

The relation between primary production and the interstitial nutrients in sediments was demonstrated by Ohle (1964) for stratified lakes. Favourable nutrient conditions in the hypolimnion and algal adaptations to conditions in the lower epilimnion maintained primary production after depletion of nitrogen and phosphorus in the epilimnion.

In shallow lakes and littoral zones the primary producers are in direct contact with the sediment. Mutual relations between primary producers in water and bacterial populations in sediment thus control to a large extent metabolism and nutrient supply from the sediment. The coupled processes of nitrification and denitrification including their interaction with phosphorus metabolism, proved to be involved in the mechanisms of nutrient recycling from the sediment. The intensity of these processes in nature is linked to external influences on the ecosystem, such as changes in nutrient supply, organic loading, electrolytic composition in tributaries and temperature.

Algal populations are characterized by rapid response to changes in the environment. During their "wax and wane", algae interact with sediment dynamics by competition for nutrients (mainly nitrogen compounds) and by contributing organic matter. This organic material partly activates the sediment metabolism and partly regulates the concentrations of elements complexed to the soluble organic pool e.g. soluble organic phosphorus.

The structure of sediment reflects previously established flow equilibria (steady states). The sediment thus counteracts spontaneous changes in ecological equilibria and thereby stabilizes the ecosystem.

Summary

Laboratory model systems consisting of various lake sediments enclosed in dialysis bags and nutrient solutions were studied with and without inoculation of the green alga *Selenastrum capricornutum*.

The ionic composition of the nutrient solutions was strongly affected by the various sediments. The initial concentrations of sodium bicarbonate in the nutrient solutions changed during the experiment due to 1) ion exchange in oxidized organogenic sediments (F, H, T), 2) oxidation reactions in reduced iron sulphide-containing sediments (SB, L), and 3) dissolution of calcium carbonate in a calcareous sediment (V) (cf. Fig. 1). pH also changed in both nutrient solutions and sediments (cf. Fig. 2). These interactions were unaffected by the presence of algae.

Nutrient transfer between sediment and solution was influenced by algae and dependent on sediment properties. Phosphorus release from the sediment was enhanced when algae were present (cf. Fig. 3). Competition for nitrate between denitrifying bacteria in the sediment and algae in the nutrient solution became more or less apparent depending on redox conditions in

the sediment (cf. Fig. 4). Ammonia, initially released to the nutrient solution, was subsequently nitrified and denitrified.

Extra additions of nutrients (nitrate or phosphate) were efficiently removed by the sediments. Nitrate was denitrified at greatly increased rates in systems with added nitrate. Phosphorus release from the sediment was suppressed when extra phosphate was added, and sorption of phosphate occurred in the reducing (V, SB) systems. Large amounts of phosphorus were recovered as soluble organic phosphorus in systems containing algae (cf. Table 8). Algal cell size was increased when sediment was present. Possible mechanisms for sediment exchange dynamics in shallow lakes and littoral zones are discussed in connection with these results.

Zusammenfassung

Wechselwirkungen von Sedimenten verschiedener Herkunft (gefüllt in Dialysesäckchen) und verschiedenen Nährlösungen die zum Teil mit *Selenastrum capricornutum* beimpft waren, wurden in Modellsystemen festgestellt.

Die Jonenzusammensetzung der Nährlösungen wurde durch die Anwesenheit der Sedimente stark beeinflusst. Bikarbonat wurde durch den Ionenaustausch in den Versuchssystemen mit oxydierenden, organogenen (F, H, T) und in den sulfidführenden Sedimenten verbraucht (SB, L), von karbonatreichem Sediment (V) dagegen abgegeben (Fig. 1). Weiters wurde das pH durch die Anwesenheit der Sedimente beeinflusst (Fig. 2).

Der Nährstoffaustausch zwischen Sediment und Lösung war von den Sedimenteigenschaften und den Algenkulturen abhängig. Die Phosphorfreisetzung von den Sedimenten wurde durch Algenkulturen gesteigert (Fig. 3). Besonders in Systemen mit reduzierenden Sedimenten konkurrierten denitrifizierende Bakterien im Sediment mit den Algen um Nitrat aus der Nährlösung (Fig. 4).

Das von den Sedimenten in den ersten Versuchstagen freigesetzte Ammonium wurde nitrifiziert und daraufhin denitrifiziert.

Nährstoffzusätze im Ueberschuss (PO_4 oder NO_3) wurden effektiv von den Sedimenten gepuffert. Nitrat wurde in erhöhtem Ausmass veratmet. Der Ausfluss von Phosphor von den Sedimenten wurde durch Phosphorzugabe unterdrückt und in den reduzierenden Sedimenten (V und SB) wurde Phosphor festgelegt. Ein Grossteil des Phosphors in Lösung wurde in den beimpften Lösungen als organisch gelöster Phosphor vorgefunden (Tabelle 8).

Das Zellgewicht und Volumen der Algen war in Systemen mit Sediment grösser als in Systemen ohne Sediment.

Auf Grund dieser Ergebnisse werden mögliche Mechanismen für die Stoffwechseldynamik in seichten Gewässern diskutiert.

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Table 1. Morphometric data on the lakes and physical-chemical characteristics of sediment, interstitial and bottom water.

BOTTOM WATER	water depth (m)	FIJOLEN		SKÄRHULTSJÖN		HINNASJÖN		TRUMMEN		S. BERGUNDASJÖN		VOMBJSJÖN		LILLESJÖN	
		area: 1.6 km ² max depth: 10 m	7	area: 0.9 km ² max depth: 14 m	13	area: 0.3 km ² max depth: 2.7 m	1.9	area: 1 km ² max depth: 2 m	2.2	area: 4.3 km ² max depth: 5.4 m	4.5	area: 12.4 km ² max depth: 15 m	13.9	area: 0.04 km ² max depth: 4.2 m	3.5
	pH	7.5	7.5	7.8	7.8	8.2	8.2	215	215	355	355	430	430	425	425
	alk meq/l	5.5	5.8	5.8	5.8	6.1	6.1	6.3	6.3	7.1	7.1	7.8	7.8	6.4	6.4
	PO ₄ -P mg/l	0.018	0.078	0.078	0.078	0.096	0.096	0.020	0.020	2.60	2.60	2.37	2.37	2.70	2.70
	tot-P "	0.005	0.008	0.008	0.008	0.008	0.008	0.020	0.020	0.020	0.020	0.048	0.048	4.20	4.20
	inorg. N mg/l	0.620	0.360	0.360	0.360	0.160	0.160	1.40	1.40	7.50	7.50	-	-	5.50	5.50
	tot-N "	0.700	-	-	-	-	-	-	-	-	-	4.10	4.10	20.0	20.0
	Fe "	0.61	2.2	2.2	2.2	0.26	0.26	1.3	1.3	1.2	1.2	0.01	0.01	22.0	22.0
	Mn "	0.049	0.29	0.29	0.29	0.19	0.19	0.4	0.4	0.9	0.9	0.02	0.02	1.8	1.8
	Ca "	3.6	5.6	5.6	5.6	2.4	2.4	18.5	18.5	26.0	26.0	73.0	73.0	26.8	26.8
	Mg "	2.1	2.2	2.2	2.2	7.1	7.1	4.3	4.3	5.5	5.5	6.8	6.8	7.3	7.3
	Na "	5.2	5.9	5.9	5.9	7.1	7.1	16.9	16.9	36.0	36.0	11.1	11.1	17.2	17.2
	K "	1.5	0.9	0.9	0.9	0.78	0.78	3.7	3.7	8.6	8.6	4.8	4.8	13.8	13.8
	SiO ₂ "	5.0	8.3	8.3	8.3	3.6	3.6	7.2	7.2	5.8	5.8	-	-	13.0	13.0
INTER-STITIAL WATER	pH	92	6.89	6.6	6.6	7.0	7.0	290	290	437	437	549	549	875	875
	alk meq/l	0.503	0.726	0.726	0.726	0.442	0.442	0.830	0.830	2.9	2.9	4.95	4.95	6.62	6.62
	PO ₄ -P mg/l	0.024	0.022	0.022	0.022	0.016	0.016	0.006	0.006	3.16	3.16	1.19	1.19	12.7	12.7
	NH ₄ -N "	0.810	4.06	4.06	4.06	1.08	1.08	1.77	1.77	11.2	11.2	6.22	6.22	66.2	66.2
	Fe "	1.67	23.6	23.6	23.6	2.5	2.5	3.07	3.07	0.26	0.26	0.12	0.12	1.58	1.58
	Mn "	0.21	0.12	0.12	0.12	1.4	1.4	3.26	3.26	1.1	1.1	1.8	1.8	31.5	31.5
	Ca "	5.5	7.9	7.9	7.9	5.35	5.35	24.6	24.6	25.4	25.4	85.0	85.0	12.1	12.1
	Mg "	1.55	2.4	2.4	2.4	1.85	1.85	5.78	5.78	5.6	5.6	16.1	16.1	58.5	58.5
	Na "	8.25	8.65	8.65	8.65	10.9	10.9	16.9	16.9	44.0	44.0	15.4	15.4	16.1	16.1
	K "	2.0	2.34	2.34	2.34	1.66	1.66	9.4	9.4	12.4	12.4	50.3	50.3	30.8	30.8
	SiO ₂ "	10.8	18.0	18.0	18.0	18.8	18.8	6.6	6.6	-	-	-	-	-	-
	pH	6.5	6.7	6.7	6.7	92.3	92.3	6.5	6.5	6.98	6.98	7.17	7.17	6.47	6.47
SURFACE SEDIMENT	dry matter g/l	71.3	60.6	60.6	60.6	296	296	79.3	79.3	143	143	116	116	51	51
	org. matter mg/gdw	338	391	391	391	296	296	337	337	185	185	219	219	563	563
	tot-P mg/gdw	2.44	1.13	1.13	1.13	1.25	1.25	1.48	1.48	5.56	5.56	1.47	1.47	0.74	0.74
	loss on ignition)	16.9	14.0	14.0	14.0	11.7	11.7	44.8	44.8	11.2	11.2	12.7	12.7	31.0	31.0
	tot-N "	36.8	34.2	34.2	34.2	51.4	51.4	46.5	46.5	25.0	25.0	19.6	19.6	21.9	21.9
	Fe "	0.38	0.61	0.61	0.61	1.28	1.28	1.17	1.17	2.06	2.06	0.73	0.73	0.62	0.62
	Mn "	4.7	4.12	4.12	4.12	3.62	3.62	5.90	5.90	6.96	6.96	182	182	8.68	8.68
	Ca "	1.7	1.09	1.09	1.09	0.88	0.88	1.38	1.38	1.56	1.56	5.27	5.27	2.62	2.62
	Mg "	0.28	0.28	0.28	0.28	0.42	0.42	0.42	0.42	0.53	0.53	0.36	0.36	1.7	1.7
	Na "	0.99	0.73	0.73	0.73	0.52	0.52	0.79	0.79	1.46	1.46	4.96	4.96	1.0	1.0
	K "	533	495	495	495	577	577	533	533	740	740	255	255	373	373
	SiO ₂ "	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TROPIC TYPES		Oligotrophic		Oligotrophic		Oligotrophic		Eutrophic		Eutrophic		Eutrophic		Eutrophic	
ADDITIONAL REFERENCE		(THUNMARK, 1931)		(ABERG & RÖDHE, 1942)		(GELIN & RIPL, 1978)		Restored 1971 (BJÖRK et al. 1972)		Eutrophicated (LEONARDSON & HENGTSSON, in press)		Eutrophicated (GELIN, 1975)		Eutrophicated Restored 1975 (RIPL, 1976)	

Table 2. Incubation conditions and design of the experimental series.

Laboratory system	Buffer added NaHCO ₃ (meq/l)	Selenastrum capricornutum (10 ⁶ cells/l)	Light conditions (4,000 lux)	Shaking (80 osc/min)	Extra nutrients added (mg/l)
Series 1	1:1 control	Selenastrum	continuous	continuous	none
	1:2	Selenastrum	continuous	continuous	none
Series 2	2:1	Selenastrum	alternating ^{x)}	alternating ^{x)}	none
	2:2	—	alternating ^{x)}	alternating ^{x)}	none
	2:3	Selenastrum	continuous	continuous	none
	2:4	—	continuous	continuous	none
	2:5	—	dark	normally twice/day	none
Series 3	3:1	Selenastrum	continuous	continuous	none
	3:2	Selenastrum	continuous	continuous	N (1.5+3)
	3:3	Selenastrum	continuous	continuous	P (0.5)

temperature for incubation: 20[±] 1°C

x) light - shaken/dark - stagnant: 16/8 hours

Table 3. Ionic composition in the nutrient solutions at the end of the experiment (15th day) in control (1:1) and in systems with NaHCO_3 added (1:2) (series 1).

System	Anions ($\mu\text{eq/l}$)				Cations ($\mu\text{eq/l}$)						
	alk.	Cl	SO_4	Σ	Ca	Mg	Na	K	H	Fe+Mn	NH_4
H, 1:1	2	43	171	216	100	35	107	9			
H, 1:2	2,810	32	297	3,139	2	1	3,175	8			
F, 1:1	2	28	572	602	362	131	89	15			
F, 1:2	3,149	26	330	3,505	5	1	3,415	13			
Sk, 1:1	0	23	1,843	1,866	1,115	242	193	32	50	144	
Sk, 1:2	1,885	27	1,386	3,298	6	1	3,219	12			
T, 1:1	0	88	645	733	384	122	148	37			
T, 1:2	2,930	72	384	3,386	5	1	3,353	18			
V, 1:1	1,730	71	2,645	4,446	3,915	216	161	36			
V, 1:2	4,303	80	1,941	6,326	1,168	149	4,959	65			
SB, 1:1	0	80	6,331	6,411	3,120	455	300	120	562	299	1,258
SB, 1:2	110	84	6,280	6,474	579	176	5,524	65			
L, 1:1	23	312	733	1,068	200	112	424	33			306
L, 1:2	1,385	317	804	2,506	3	1	2,349	25			133
Z8, 1:1	15	13	31	59	7	4	43	10			64
Z8, 1:2	5,510	6	38	5,554	3	1	5,525	11			5,540

Table 4. Total phosphorus and algal yield in the nutrient solutions on the 7th and 15th day of the experiment for systems with NaHCO_3 added (series 1).

System	Total phosphorus, $\mu\text{g/l}$		Algal yield, 10^6 cells/l	
	7th day	15th day	7th day	15th day
F, 1:2	144	525	105	2000
Sk, 1:2	8	60	40	70
T, 1:2	50	210	40	1450
V, 1:2	8	13	70	120
SB, 1:2	59	127	55	1300
L, 1:2	207	720	1060	6100

Table 5. Ionic composition and conductivity in the nutrient solutions at the end of the experiments (15th day) (series 2 and 3).

System	Anions ($\mu\text{eq/l}$)				Cations ($\mu\text{eq/l}$)				Conductivity ($\mu\text{S/cm}$)	
	alk.	Cl	SO ₄	Σ	Ca	Mg	Na	K	Σ	initial final
F, 2:3	383	18	382	793	1	1	792	4	798	98 77
T, 2:3	75	60	382	817	2	1	809	9	821	79
V, 2:3	1,728	25	2,355	4,108	2,824	137	1,131	13	4,108	372
SB, 2:3	0	42	2,628	2,670	1,093	192	1,209	39	2,533	276
Z8, initial	1,208	12	24	1,208	1	1	1,166	1	1,169	98
H, 3:1	840	34	327	1,201	12	3	1,270	4	1,289	190 113
F, 3:1	1,064	63	357	1,484	5	0	1,470	10	1,485	132
T, 3:1	936	53	335	1,324	4	0	1,357	12	1,373	128
V, 3:1	2,084	36	2,724	4,844	2,545	152	2,284	20	5,001	415
SB, 3:1	77	77	2,603	2,757	419	116	2,197	39	2,771	286
Z8, initial	2,242	24	7	2,273	7	4	2,392	3	2,406	190

Table 6. Phosphorus and nitrogen transfer rates through the dialysis membrane. Positive transfer rates indicate an outflow from the sediment bags, while negative transfer rates refer to an inflow to the sediment.

System	Phosphorus transfer rates (mg/m ² day)		Nitrate-N transfer rates (mg/m ² day)	
	max (on day)	average (15 days)	0-4th day (1st addition)	10-12th day (2nd addition)
F, 3:1 (control)	5.1 (11th)	2.1		
F, 3:2 (+ nitrogen)	7.0 (11th)	2.3	- 14	- 63
F, 3:3 (+ phosphorus)	2.1 (9th)	0.4		
H, 3:1 (control)	1.7 (11th)	0.4		
H, 3:2 (+nitrogen)	2.6 (9th)	0.6	- 9	- 20
H, 3:3 (+phosphorus)	1.4 (14th)	- 0.2		
T, 3:1 (control)	6.2 (13th)	2.2		
T, 3:2 (+ nitrogen)	4.2 (11th)	1.0	- 12	- 10
T, 3:3 (+ phosphorus)	2.7 (9th)	0.3		
V, 3:1 (control)	1.7 (11th)	0.3		
V, 3:2 (+ nitrogen)	2.9 (11th)	0.5	- 20	- 93
V, 3:3 (+ phosphorus)	0.9 (11th)	- 1.1		
SB, 3:1 (control)	2.9 (11th)	1.0		
SB, 3:2 (+ nitrogen)	5.8 (11th)	1.5	- 19	- 92
SB, 3:3 (+ phosphorus)	0.4 (14th)	- 0.9		

Table 7. Maximum specific growth rate and final algal yield for *Selenastrum capricornutum* in the experimental systems (series 3).

System	Maximun specific growth rate, μ /day*		
	control	+ nitrogen	+ phosphorus
F, 3	0.80	0.90	1.28
H, 3	0.69	0.69	1.30
T, 3	0.81	0.55	1.22
V, 3	1.24	1.42	1.10
SB, 3	0.90	1.15	1.35

$$*\mu = \frac{\ln(X_{n+1}) - \ln X_n}{t}$$

System	Final algal yield, 10^6 cells/l		
	control	+ nitrogen	+ phosphorus
F, 3	1 250	1 780	880
T, 3	600	—*	2 740
V, 3	290	290	960
SB, 3	320	1 500	840

* terminated earlier due to infection by other organisms

Table 3. Phosphorus fractions in sediment at station 10 at the end of the experiment (Series 3).

System	PO ₄ -P		Particulate P		Soluble organic P		Total P
	µg/l	%	µg/l	%	µg/l	%	µg/l
F, 3:1 (control)	20	4.6	178	40.9	237	54.5	435
F, 3:2 (+ nitrogen)	10	2.1	115	23.7	360	74.2	485
F, 3:3 (+ phosphorus)	150	26.8	260	46.4	150	26.8	560
T, 3:1 (control)	< 5	<1.1	70	15.7	370	83.1	440
T, 3:2 (+ nitrogen)	10	4.2	61	25.4	169	70.4	240
T, 3:3 (+ phosphorus)	20	3.8	327	61.7	183	34.5	530
V, 3:1 (control)	< 5	<6.3	8	10.0	67	83.7	75
V, 3:2 (+ nitrogen)	< 5	<3.6	4	2.9	131	93.6	135
V, 3:3 (+ phosphorus)	< 5	<2.0	145	59.2	95	38.8	240
SB,3:1 (control)	20	8.7	43	18.7	167	72.6	230
SB,3:2 (+ nitrogen)	< 5	<1.5	73	22.1	252	76.4	325
SB,3:3 (+ phosphorus)	< 5	<1.6	172	53.7	143	44.4	315

Table 9. Dry weight and volume of *Selenastrum capricornutum* grown in different sediment-nutrient solution systems (series 3) and in a 5% Z8 nutrient solution.

System		Dry weight		Variance		Dry weight		Variance		Volume	
		$\mu\text{g}/10^6$ cells		%		$\mu\text{g}/\mu\text{g P}$		%		$\frac{\text{mm}^3}{10^6 \text{ cells}}$	
Average, 3:1	(control)	37		16		326		31		0.09	
Average, 3:2	(+ nitrogen)	46		6		774		25		0.12	
Average, 3:3	(+ phosphorus)	19		27		102		28		0.05	
Mean values (3:1,3:2 & 3:3)		31		40		333		86			
5% Z8		19				380				0.056	

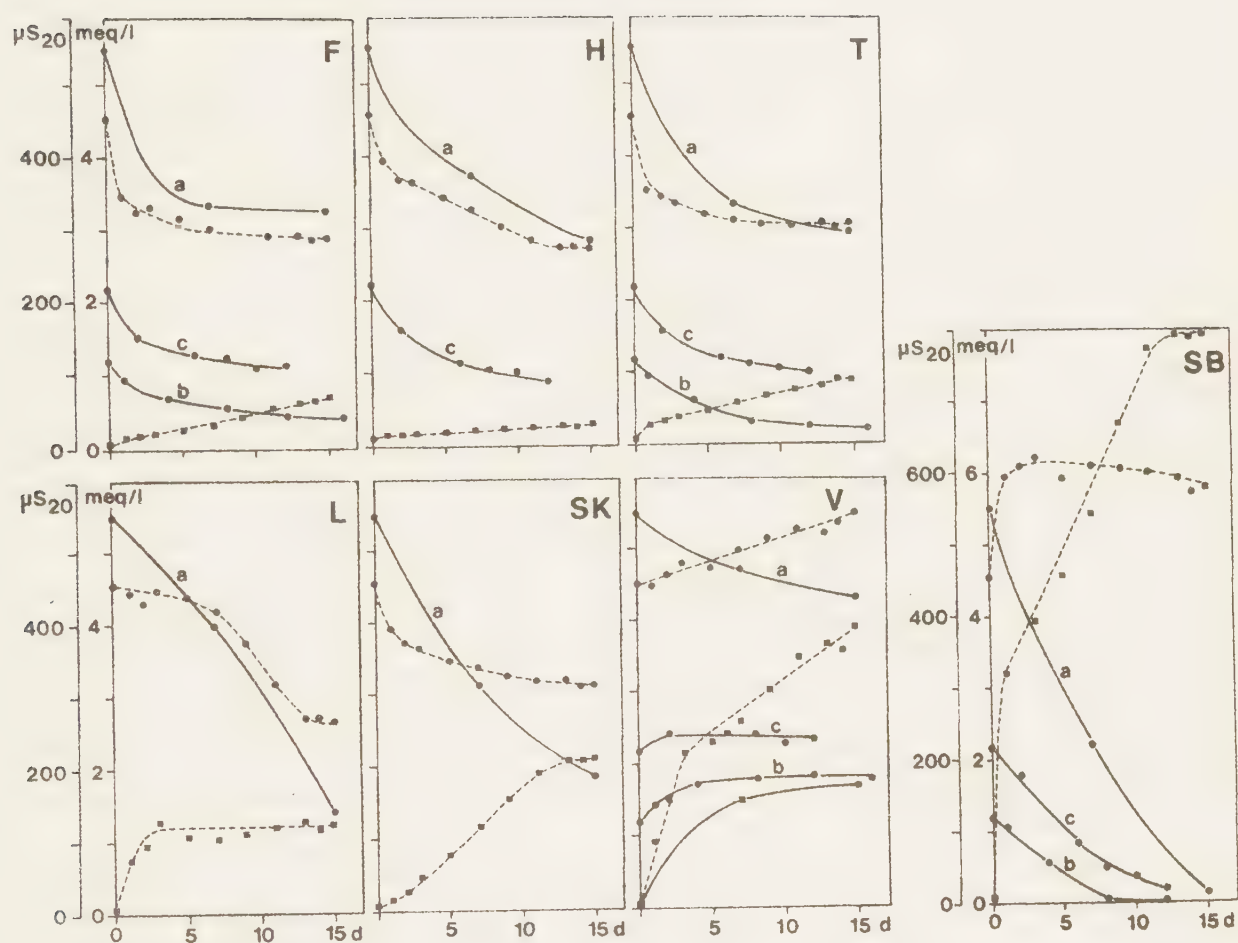


Fig. 1. Alkalinity (—) and conductivity (---) as functions of time in the nutrient solutions incubated with sediment and algae. Alkalinity (adjusted with NaHCO_3) (—●—) was initially 5.5(a), 1.2(b) and 2.2(c) meq/l for series 1, 2 and 3, respectively. Conductivity (---●---) was initially $455 \mu\text{S}_{20}/\text{cm}$ for series 1. Alkalinity (—■—) and conductivity (---■---) without NaHCO_3 were initially 0.015 meq/l and $7 \mu\text{S}_{20}/\text{cm}$, respectively.

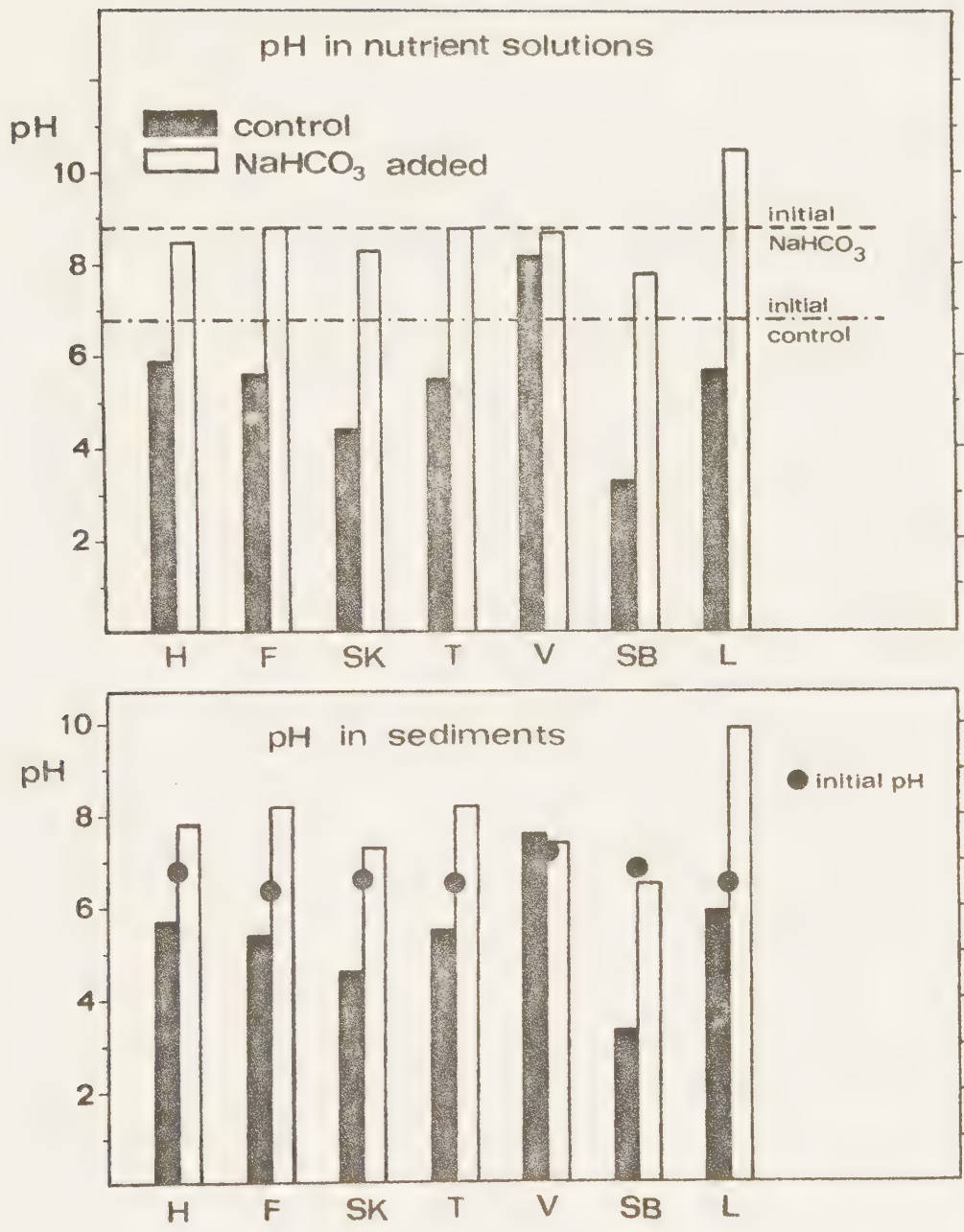


Fig. 2. Initial and final pH in nutrient solution and sediment (series 1).

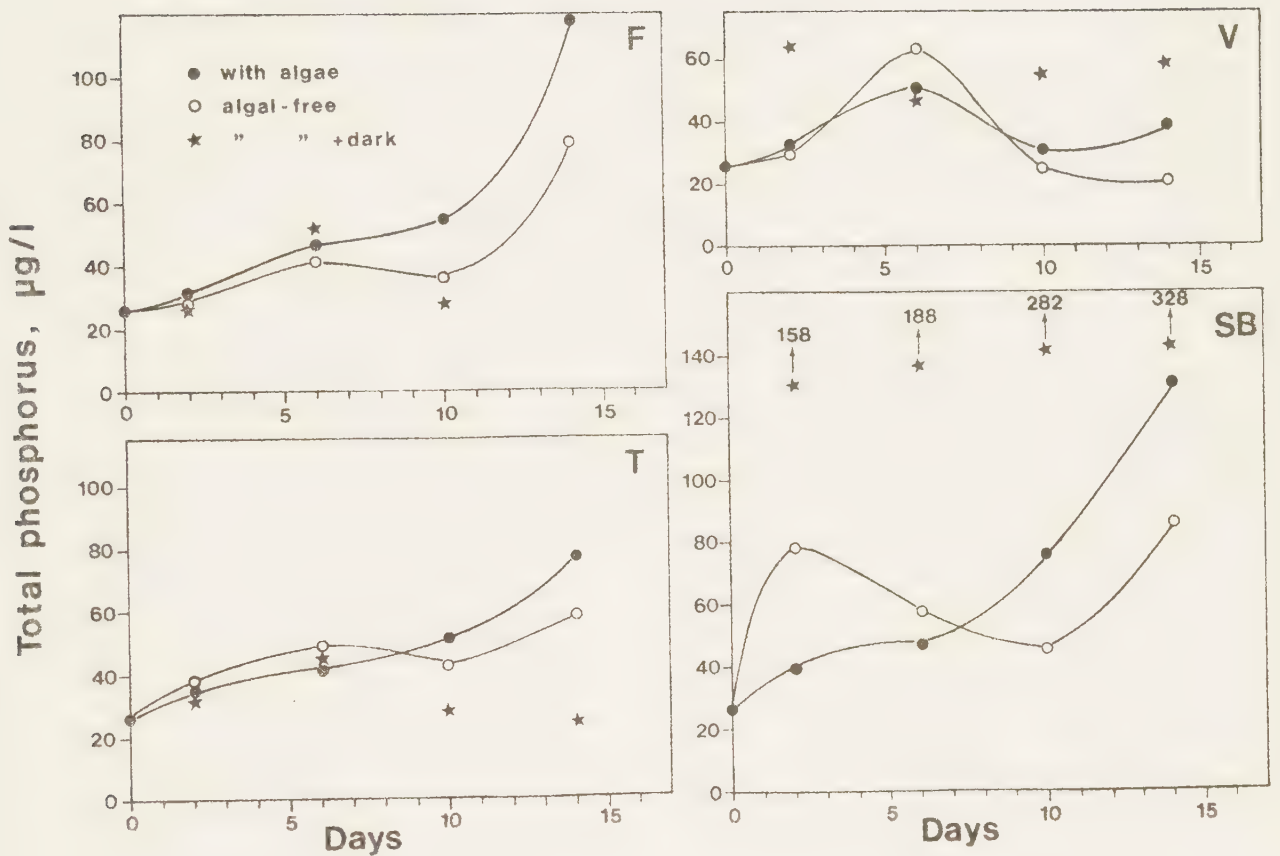


Fig. 3. Total phosphorus in sediment-nutrient solution systems incubated with and without algae in the light and without algae in the dark (series 2).

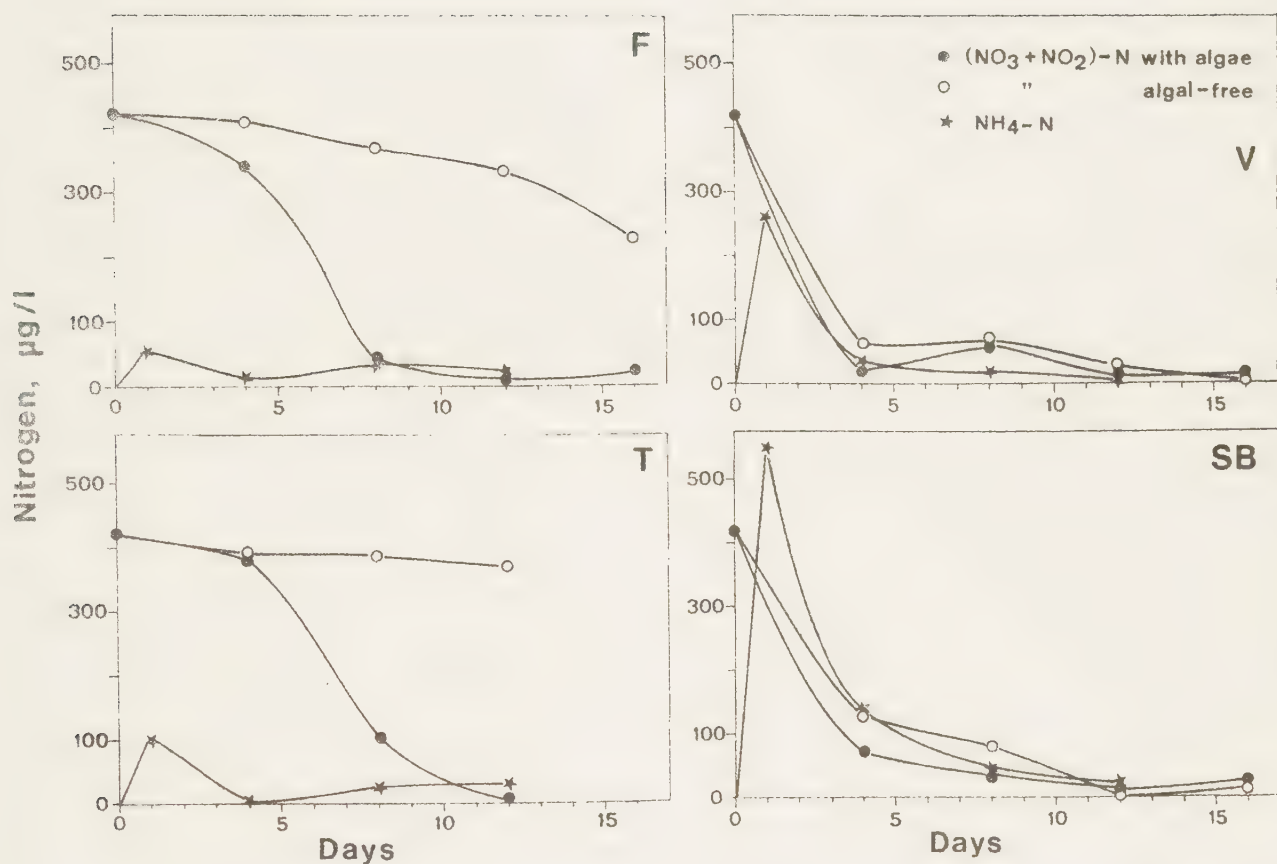


Fig. 4. Inorganic nitrogen in sediment-nutrient solution systems incubated with and without algae. Ammonia concentrations represent mean values of all experimental systems (no significant differences between the individual systems were observed) (series 2).



LIMNOLOGISKA INSTITUTIONEN LUNDS UNIVERSITET

EFFECTS OF ENVIRONMENTAL STRESSES ON THE RELATIONSHIP BETWEEN *PLECTONEMA BORYANUM* AND CYANOPHAGE LPP-1

Gunilla Lindmark

INSTITUTE OF LIMNOLOGY
University of Lund

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EFFECTS OF ENVIRONMENTAL STRESSES ON THE RELATIONSHIP BETWEEN
PLECTONEMA BORYANUM AND CYANOPHAGE LPP-1

By Gunilla Lindmark

Institute of Limnology, University of Lund,
Lund, Sweden.

INTRODUCTION

For many years a reduction of nutrient input to lakes was considered the universal remedy to combat the cultural eutrophication created during the last decades. Although meaningful, the measures were not sufficient in several cases (Björk et al. 1972, Ryding & Forsberg 1976), and additional alternative approaches are in demand (Shapiro 1977, 1978).

Changing phytoplankton communities from blue-green algal dominance to green algae with the intention of improving the algal food resource for higher organisms in the ecosystem is an attractive approach which was successfully accomplished by Shapiro (1973) and Shapiro et al. (1975). In their experiments, performed on a small scale in the field (plastic enclosures), a rapid shift from blue-green algae to green algae followed an injection of CO₂ and the accompanying drop in pH. Whether the supply of CO₂ favoured the green algae in competition with blue-green algae for carbon, or whether other organisms were affected and involved in the mechanism of the shift is still unclear. Artificial circulation of whole lakes has sometimes resulted in a shift from blue-green to green algae or diatoms.

The successful ventures generally were accompanied by a lowering of pH (Shapiro 1977).

The recent attention focused on the microbial pathogens of blue-green algae and their interrelations has exposed an attractive field where many questions remain despite intense research efforts (Safferman & Morris 1963, Stewart & Brown 1969, Safferman 1973b, Padan & Shilo 1973, Daft et al. 1975, Stewart & Daft 1977). Several investigators (Safferman & Morris 1964, Jackson & Sladecek 1970, Cannon et al. 1974, Cannon 1975) have commented on the use of viruses as a means of controlling algal blooms. However, Goryushin (1976) warns for the ability of cyanophages to induce mutation in higher organisms. Nevertheless, the presence of cyanophages in environments common to many blue-green algae, e.g. at high pH, must be significant as a selective factor.

The present study is an attempt to clarify whether relations between viruses (cyanophages) and their algal hosts can be affected by manipulations in the environment. Is it possible to activate cyanophages and accelerate lysis of blue-green algal populations or to enhance the resistance of blue-green algae to attack from cyanophages?

The experiments presented here were performed under laboratory conditions with a well-known algal - cyanophage system, *Plectonema boryanum* and cyanophage LPP-1 (attacking strains of *Lyngbya*, *Phormidium* and *Plectonema*). The work was done in close connection with field experiments on natural blue-green algal communities, however, because the nature of the induced blue-

green algal collapse in plastic enclosures suggested lysis of the algal cells (Shapiro et al. 1975, and pers. commun.).

The rate of LPP-1 cyanophage replication and lysis of *Plectonema* was studied in relation to:

- a) pH alterations by CO₂/air additions
- b) algal host culture age and density
- c) nutrient concentrations
- d) presence of additional algal species

The results will be discussed in relation to observations from field experiments (Shapiro 1973, Shapiro 1977).

MATERIALS AND METHODS

Algal and Cyanophage Strains

The filamentous blue-green alga, *Plectonema boryanum* IU 581 (Indiana Univ. Culture Collection) and its cyanophage LPP-1 (kindly supplied by Dr. R.E. Cannon at University of North Carolina, Greensboro) were the main organisms selected for the experiments. Morphological and physicochemical characteristics of the LPP cyanophage and its algal hosts and the process of viral infection are reviewed by Padan & Shilo (1973).

Additional algal strains used were cultures of the blue-green alga *Anabaena circinalis* (Wis 1038) and the green alga *Scenedesmus* sp. (probably *S. obliquus*, Rhee 1972).

Culture Methods

Algal stock cultures were grown in a synthetic nutrient solu-

tion (modified Gorham's medium, Table 1), and algal cells were routinely transferred into fresh nutrient solution at intervals of 10 days. Cells from 12-day old stock cultures (density ca. $20 \cdot 10^6$ cells/ml) were used as inoculum in the experiments. Both stock and experimental algal cultures were incubated at $24 \pm 2^\circ\text{C}$ and were constantly illuminated by cool-white fluorescent lamps with an intensity of 1500 lux. The stock cultures were hand shaken once daily while cultures in the experimental flasks were mixed for 7 minutes once an hour by teflon-coated magnetic stirring bars.

Cyanophage suspensions were obtained by lysis of a host algal culture and filtered (Millipore $0.22 \mu\text{m}$). The suspensions were stored refrigerated and in the dark to prevent losses in viability (Padan & Shilo 1973). The titer of the cyanophage suspension was assayed prior to every experimental series. The requirement of the LPP-1 cyanophage for 10^{-3} M MgCl_2 in the medium was fulfilled in all experimental cultures.

The experiments were run as batch algal cultures with 500 ml of nutrient solution in 1-liter Erlenmeyer flasks. The flasks with nutrient solution were autoclaved with two Pasteur pipets inserted through the cotton plug and with tygon tubing attached to one of the pipets for sampling (cf. Fig. 1). Sampling was performed by inserting sterilized Pasteur pipets into the tubing and removing culture suspension by suction immediately after mixing. No attempts were made to keep the cultures completely free from bacteria. Depending on the design of the experiment, the cultures were incubated for 8 to 25 days. Due

to limited experimental culture volumes and the time factor in viral replication, it was necessary to set priorities in the analytical program to give maximum information in a limited number of experiments.

Description of a Culture Unit

A schematic diagram of one culture unit is shown in Fig. 1. In each experimental series six or seven culture flasks with separate pH meters and valves for CO₂/air (5:95, v/v) were connected in parallel to a timer and the CO₂/air tank. The pH meters were operating constantly during the experiment and functioned to hold pH levels below preset values. The timer activated stirring of the culture solutions and opened the valves for CO₂/air bubbling seven minutes per hour. The valves were secondarily controlled by pH in the cultures, and gas was provided if pH exceeded the value set on the pH meter for a particular culture. As soon as pH levels were corrected the valves were closed. The tubing for gas supply was transparent tygon and glass when possible. Due to a pressure decrease from loss of gas by diffusion through the walls of the tubing when no gas was required for a longer time (ca. 5-6 hrs), culture solution was sucked up into the inlet pipet and occasionally into the tubing. Various kinds of tubing were tested but none worked satisfactorily. Therefore, either the gas supply mechanism was interrupted during the night or the outlets (pipet tips) were raised above the surface of the culture suspension. In the latter case the gas supplied was not always enough

for complete correction of pH increases in the cultures.

Analytical Methods

Physical and chemical analyses

pH was recorded every second to third hour during daytime.

$\text{PO}_4\text{-P}$ was analysed as soluble reactive phosphorus according to Murphy & Riley (1962) and by the stannous chloride method.

$\text{NH}_4\text{-N}$ was determined according to Chaney & Marbach (1962), and

$\text{NO}_3\text{-N}$ was analysed as $\text{NO}_2\text{-N}$ (Bendschneider & Robinson 1952) after reduction by a copperized Cd column.

Chlorophyll a

5-25 ml of algal suspension was filtered under vacuum on a 2.5 cm glass fiber filter (Whatman GF/C) and washed with a small volume of distilled water. The filter was ground in 90 % acetone, and chlorophyll was extracted for 12 hrs in the dark at $4\text{-}6^\circ\text{C}$. Estimation of chlorophyll a followed the method of Lorenzen (1967).

Cyanophage assays

For plaque assays, 0.5 ml portions of diluted viral suspension were mixed with 2.0 ml of a dense algal host culture and 2.5 ml of melted nutrient agar (nutrient solution in 1 % agar) and plated on Petri dishes over a base of 15 ml solidified nutrient agar (nutrient solution in 1.5 % agar) according to the double-agar layer technique (Safferman 1973a). The plates were inverted and incubated under continuous illumination (1500 lux) at $22\pm 1^\circ\text{C}$, and the number of cyanophage particles was estimated by counting clear plaques on the algal "lawns" after 4-5 days incubation.

Appropriate cyanophage titer for assay was obtained through tenfold dilutions in a saline-magnesium solution (0.2 g $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ and 5.85 g NaCl per liter distilled water).

Each plaque developed on the plate was considered to originate from one cyanophage, and the titer of the assayed suspension (plaque-forming units (PFU)/ml) was evaluated using the proper dilution factor.

EXPERIMENTAL DESIGN AND RESULTS

The experiments were performed in four successive series with six or seven culture flasks in each series. Results from one series aided in determining of the next. Since the addition of CO_2 /air to reduce pH did not show any differences in the nature or rate of lysis of *Plectonema* cultures when compared to the use of HCl in a preliminary study, addition of CO_2 /air was selected for all experiments to avoid changes in alkalinity (Stumm & Morgan 1970, Brewer & Goldman 1976).

Series 1

This experiment was done to determine whether pH has an effect on the alga-cyanophage relationship. *Plectonema* was grown in the nutrient solution (at 1 mg $\text{PO}_4\text{-P/l}$) for 11 days with no pH adjustment. The cyanophage was then inoculated, and pH adjustment with CO_2 /air was begun (Fig. 2). Controls received no CO_2 /air.

Culture no.	pH adjusted with CO ₂ /air to:	LPP-1 added (PFU/ml)
1	5.5	10 ⁵
2	6.5	10 ⁵
3	7.5	10 ⁵
4	control	10 ⁵
5	5.5	0
6	control	0

When pH was lowered from 11.5 to about 7.5 (Fig. 2) in cultures of *Plectonema* simultaneously inoculated with cyanophage, replication of the cyanophage was strongly enhanced (Fig. 3). In the culture where pH was lowered to 5.5, replication was delayed. After three days of incubation, however, the cyanophage concentration was similar to that in the other cultures with pH adjustment. In the culture where pH was not adjusted (Fig. 2, culture 4) on the other hand, replication of the cyanophage declined early and ceased at a comparably lower density (Fig. 3).

The response of *Plectonema* to the presence of the cyanophage was obvious after only 24 hours (Fig. 4) in cultures where CO₂/air was supplied and the pH lowered, while algae in the control culture (without CO₂/air addition) maintained a healthy population despite an increase in cyanophage particles of several orders of magnitude (Fig. 4, cf. Fig. 3).

Plectonema cultures free from cyanophage showed the same healthy growth irrespective of the change in pH (Fig. 4). The only difference noted between the two cyanophage-free cultures was

that the algal filaments became short (5-30 cells) and straight in the culture with adjusted pH, while filaments in the control culture were long (>30 cells) and twisted into bundles. According to Padan & Shilo (1973), however, the length of the filaments has no effect on the adsorption rate of cyanophage to *Plectonema* or the reproductive cycle of the cyanophage.

Two days after the viral infection of *Plectonema*, only one- and two-cell trichomes were left in the cultures with adjusted pH, but long (>20 cells) and bright green trichomes were present in the control culture. The only symptom of viral infection in the control was that the cells swelled somewhat and were thus abnormally distinct in the filaments. After four days there were no detectable *Plectonema* cells in cultures with adjusted pH and cyanophage. However, 17 days after the infection (adjustment of pH stopped on day 6) some long, fine filaments were observed in the lysed cultures indicating regrowth of a "new" *Plectonema* population. pH had by then increased to about 9. This behaviour can be compared with the repeated oscillations in *Plectonema* density and cyanophage LPP-1 concentration observed in continuous cultures (Cowlshaw & Mersa 1975, Cannon et al. 1976).

When the addition of CO₂/air was interrupted during the night, pH increased to the same levels in cultures with cyanophage as in cyanophage-free cultures (Fig. 2). In control cultures (no CO₂/air addition) the presence of the cyanophage did not affect pH conditions during the experiment (Fig. 2, cultures

4 and 6). According to Padan et al. (1970), cyanophage infection begins to inhibit photoassimilation in *Plectonema* cultures early in the infective process. Wu & Shugarman (1967), however, stated that the rate of photosynthetic O_2 evolution does not change until the infected cells rupture.

Thus, the present results show clearly that although the alga can live at low pH, and the alga and cyanophage can coexist at high pH, lysis occurs when the pH is lowered in an infected culture.

Phosphate concentrations were below $10 \mu g PO_4-P/l$ in all systems throughout the experiment (i.e. for 6 days after cyanophage inoculation).

Series 2

The purpose of this experiment was to repeat parts of series 1 using a more dilute algal culture and to determine whether continuous pH adjustment is necessary to cause lysis of the host alga. In addition, a more dilute medium was used. Fewer cyanophage particles were added to give a number pf phage particles per algal cell similar to that in series 1.

Plectonema cells (35 ml of a 12-day old stock culture) were inoculated into a half-strength ($0.5 mg PO_4-P/l$) nutrient solution (Table 1). This was stirred 3 h for pH stabilization before cyanophage LPP-1 was inoculated and CO_2 /air addition begun (Fig. 5). In two cultures CO_2 /air injections were performed only one and three times, respectively.

Culture no.	pH adjusted with CO ₂ /air to:	LPP-1 added (PFU/ml)
1	6	$2 \cdot 10^3$
2	7	$2 \cdot 10^3$
3	6 (3 times)	$2 \cdot 10^3$
4	6 (once)	$2 \cdot 10^3$
5	control	$2 \cdot 10^3$
6	6	0
7	control	0

In this series the fastest replication of cyanophage occurred in the culture where no CO₂/air was added (Fig. 6, control culture). Because of the dilution of the algal inoculum, however, pH values in the cultures were more than 2 pH units lower than in the first series at the time the cyanophage was inoculated (Fig. 5, cf. Fig. 2). Furthermore, pH was controlled by cyanophage activity after one day of incubation, since photosynthetic activity was affected (Fig. 5). In the cyanophage-free control culture pH increased as a function of algal growth (Fig. 5, cf. Fig. 7).

In the cultures with pH adjustment cyanophage replication was delayed, but it later progressed at a similar rate in all cultures (Fig. 6). As in the earlier experiment (series 1), the "most acid" culture (pH ~6) contained a lower concentration of cyanophage during the first two days of viral infection than did the cultures where pH was kept around 7 (Fig. 6).

Chlorophyll a development in the cultures was inversely related to cyanophage replication (Fig. 6 and 7).

The rapid lysis of *Plectonema* cells in the control culture (no CO₂/air addition) directly reduced the uptake rate of PO₄-P (Fig. 8, culture 5). Cultures with one or three injections of CO₂/air were able to absorb more PO₄-P before being affected by cyanophage activity (Fig. 8, culture 3 and 4). In the cultures with pH adjustment and cyanophage, only small amounts of PO₄-P were left in solution, and only a slight increase in PO₄ could be seen on about the 7th day of the infection period (Fig. 8). The concentration of phosphate was initially 500 µg P/l; however, three hours after addition of algal cells, i.e. at the time for cyanophage inoculation, only 200 µg PO₄-P/l remained in solution. The cyanophage-free cultures depleted PO₄-P from the nutrient solution within three days (Fig. 8).

Two days following cyanophage inoculation NH₄-N appeared in solution in some cultures (Fig. 8). Since the culture which lysed first showed the earliest indication of NH₄-N, this was probably connected to cyanophage activity. No NH₄-N was found in uninfected cultures (Fig. 8). Cultures with frequent input of CO₂/air showed a rapid increase to high concentrations of NH₄-N, most probably due to algal lysis. The nitrogen source in the nutrient solution was NO₃, and the N:P ratio (by weight) was 80:1.

The replication rate of cyanophage in the cultures was not affected by making the CO₂/air injections once, three times or frequently. With respect to PO₄-P and NH₄-N release following lysis, on the other hand, the frequency of CO₂/air addition was critical (cf. Fig. 8).

Series 3

Because of the differences in results between series 1 and 2, i.e. the virus replicated more at ambient pH in series 2, the algal cultures used in this series were again older and more concentrated than in series 2.

Plectonema (10 ml inoculum) was allowed to grow in a half-strength nutrient solution modified to 5 mg $\text{NO}_3\text{-N/l}$ and 0.35 mg $\text{PO}_4\text{-P/l}$ for 4.5 days before cyanophage was inoculated and $\text{CO}_2\text{/air}$ addition was started (Fig. 9). In this series 1 ml of 12-day old stock cultures of *Scenedesmus* or *Anabaena* was added simultaneously with the cyanophage inoculation.

Culture no.	pH adjusted with $\text{CO}_2\text{/air}$ to:	LPP-1 added (PFU/ml)	Additional alga
1	6	10^4	-
2	6	10^4	<i>Scenedesmus</i>
3	6	10^4	<i>Anabaena</i>
4	6	0	-
5	control	10^4	-
6	control	10^4	<i>Scenedesmus</i>
7	control	10^4	<i>Anabaena</i>

The replication of cyanophage was much slower in these cultures than when the host algae had approached a stationary phase of growth (Fig. 10, cf. Fig. 3) or when a surplus of inorganic nutrients was present in the culture (cf. Fig. 6). As in series 1 where no external nutrients were present, a faster replication of cyanophage LPP-1 occurred in the cultures with pH adjustment. In addition, pH levels in the cul-

tures were similar to those in series 1 at the time of cyanophage inoculation (Fig. 9, cf. Fig. 2).

Anabaena or *Scenedesmus* added to the *Plectonema* cultures simultaneously with cyanophage in this series did not significantly affect the cyanophage replication pattern (Fig. 10). Nor did chlorophyll a concentrations in the cultures show any differences during the first three days that could be related to algal species composition (Fig. 11). The main differences were due to the CO₂/air addition and the related variation in cyanophage activity.

Neither PO₄-P nor NH₄-N could be detected during the first three days of infection. On the 8th day, however, 210 µg/l and 190 µg/l of NH₄-N were found in the cultures with pH adjustment containing *Plectonema* and *Plectonema* + *Anabaena*, respectively. On the other hand, no NH₄-N was found in the culture with pH adjustment containing *Plectonema* + *Scenedesmus* or in any of the cultures where pH was high. NO₃-N and NO₂-N were below 25 µgN/l and 10 µgN/l, respectively, in all cultures. Initial input of NO₃-N and PO₄-P was much lower in this series, i.e. 5 mg N/l and 0.35 mg P/l.

After two days of incubation with cyanophage, *Plectonema* consisted of short (3-10 cells) filaments in the cultures with pH adjustment; *Scenedesmus* looked healthy, while only a small number of *Anabaena* cells remained viable. In the control cultures (no CO₂/air addition) the *Plectonema* filaments after two days were long (>30 cells) and twisted into bundles when

no additional algal species were present, and long, but not twisted, when *Scenedesmus* or *Anabaena* was present. After four days of incubation, when *Plectonema* in the control cultures consisted of 1-3 cell filaments and lysis was in progress, *Anabaena* in particular looked very healthy. *Scenedesmus* was more abundant in the culture with pH adjustment than in the control culture. pH in the control cultures decreased after 3 days of incubation (Fig. 9). Small amounts of bacteria were found in the lysed cultures after eight days of incubation.

Series 4

Since the age of the algal host culture seemed to affect the rate of cyanophage replication, identical nutrient solutions inoculated with *Plectonema* cells were allowed to reach different growth phases (considered as host age), and different densities and pH (Fig. 12) before cyanophage LPP-1 was inoculated.

Plectonema (10 ml inoculum) was allowed to grow in a half-strength nutrient solution modified to 3 mg $\text{NO}_3\text{-N/l}$ and 0.3 mg $\text{PO}_4\text{-P/l}$ for 1.5, 6 and 11 days before cyanophage inoculation and the start of CO_2 /air addition (Fig. 12).

Culture no.	pH adjusted with CO_2 /air to:	LPP-1 added (PFU/ml)	Algal host culture age (days)
1	7	10^3	1.5
2	control	10^3	1.5
3	7	10^3	6
4	7 (once)	10^3	6
5	7	10^3	11
6	8 (once)	10^3	11

As found in earlier experiments, the cyanophage attacking algae in the exponential phase of growth showed the slowest rate of replication (Fig. 13). Again, algae in the lag phase of growth induced a rapid increase in cyanophage, indicating that variation in algal density was less important for viral replication than the physiological state of the host. The chlorophyll a concentration was 2.6 and 3.2 times higher after 6 and 11 days of initial incubation when compared with the value after 1.5 days.

The difference in cyanophage replication between one or frequent additions of CO₂/air was much smaller than that caused by algal host age (Fig. 14). Again, the infection of the "youngest" *Plectonema* culture resulted in a release of NH₄-N (250 µg/l on day 10 and 510 µg/l on day 15) in the culture with pH adjustment. PO₄-P was below 5 µg/l up to day 10 in all cultures, but on day 15 it ranged between 10-21 µg PO₄-P/l with no relation to host culture age or CO₂/air input.

DISCUSSION

Since Safferman & Morris (1963) isolated the first cyanophage, designated LPP since it lyses species of the blue-green algal genera *Lyngbya*, *Phormidium* and *Plectonema*, additional cyanophages have been discovered and investigated (cf. reviews by Padan & Shilo 1973, Stewart & Daft 1977). The main emphasis has been on cyanophage characteristics, growth cycles and interactions with host algal metabolism, mostly under standard photosynthetic conditions (Wu & Shugarman 1967, Padan, Ginzburg

& Shilo 1970, Padan & Shilo 1973, Stewart & Daft 1977). It is clear that viral replication within the algal host is regulated largely by host metabolism, and that viral infection in turn affects the physiological state of the host alga.

Interpretation of the results from this study will be centered on how a) input of CO_2 /air and accompanying changes in pH, b) inorganic nutrients, and c) physiological state of the host alga (host culture age) affected the replication of the cyanophage LPP-1 in *Plectonema boryanum* cultures. As the conditions simulated in the laboratory were to a large extent derived from treatments used in plastic bag experiments (Shapiro 1973, 1975), the laboratory results are compared with the outcome of field experiments. Only those cyanophage - alga interactions that are more or less directly related to the areas defined above will be considered.

Dependence on pH and CO_2 Input

Since growth of blue-green algae frequently occurs under high pH conditions (King 1970), and collapse can be induced by a lowering of pH (Shapiro 1973), it was interesting to find that *Plectonema* continued to grow at high pH levels where lysis was avoided, even though the cyanophage concentration initially increased. On the other hand, the addition of CO_2 /air to a culture containing the cyanophage induced a rapid lysis of the *Plectonema* population despite the fact that the alga could grow well at low pH in the absence of the cyanophage.

LPP cyanophage activity is reported to be stable from pH 5 to 11 (pH adjusted with HCl or NaOH in filtered viral suspensions) by Safferman & Morris (1964). The same authors reported that *Plectonema* develops readily at pH 7-11, whereas no, or poor growth occurs below pH 7 (cf. Padan & Shilo 1973). In my experiments the growth of *Plectonema* was not particularly affected when pH was lowered by addition of CO₂/air to as low as 5.5 or allowed to rise to nearly 12. Instead, high (>11) and very low (5.5-6) pH values seriously affected cyanophage replication, and the reduced or delayed infection of the algal cells could favour algal growth and development of chlorophyll a (cf. Fig. 4 and 7). Between pH 6 and 11 there were no significant variations in cyanophage replication patterns that could be related to pH conditions solely. According to Padan et al. (1970), replication of cyanophage LPP-1G was identical in the presence or absence of CO₂.

In lysed cultures with no pH adjustment, pH stabilized between 8 and 9. Addition of Na₂CO₃ to lysed cultures to raise the pH accelerated a regrowth of *Plectonema*, while addition of nutrient solution had no effect on regrowth (Lindmark unpubl.). Attempts to isolate *Plectonema* from sites where LPP cyanophages were found have proved fruitless (Cannon et al. 1974).

In field experiments (plastic bags) the induced collapse of the blue-green algal populations and the shift in species dominance occurred at pH values as high as 8.5 (Shapiro 1975). Furthermore, a reversal of the shift (to blue-greens) required re-inoculation of blue-green algae as well as increase in pH (Shapiro op. cit.).

LPP cyanophages naturally occur mostly in polluted environments, e.g. waste stabilization ponds, fish ponds during periods of fish kill, polluted rivers and rice fields (cf. Safferman & Morris 1967, Shane et al. 1972, Singh 1973, Cannon et al. 1974). Host algae have generally not been observed in samples that showed a presence of LPP virus (Safferman & Morris op. cit.). For example, along the polluted Christina River (in Delaware, U S A), host algae were found only occasionally in samples where LPP cyanophages were present. A virus-resistant *Plectonema* laboratory culture grew well in the river water. However, the river pH was reported to be relatively stable, varying between 6.6 and 7.8, and concentrations of inorganic N and $\text{PO}_4\text{-P}$ were high (Shane et al. 1972). Similar observations were made in a pond close to Minneapolis (Minnesota, U S A) (Lindmark unpubl.). Survival and tolerance of the LPP cyanophages apparently enable them to control their algal hosts in nature and thus eliminate the development of blooms.

Other cyanophages discovered later have been found to possess somewhat different requirements, e.g. the SM-1 cyanophage (attacking strains of *Synechococcus*) requires CO_2 for replication (cf. Stewart & Daft 1977). Nevertheless, additional cyanophages need to be isolated and characterized. Just as there are specific bacteriophages for a large number of bacteria, it seems possible that there is a cyanophage for each blue-green alga.

Dependence on Inorganic Nutrients

Whether the addition of nutrients or the associated decline in pH (from 11.5 to ca. 9) favoured cyanophage replication in the control culture (no addition of CO₂/air) in series 2 as shown in Fig. 6, is unclear. The inoculation of cyanophage in relation to algal density was about one third of that used in experiments where no nutrients were added before cyanophage inoculation. In spite of this, the amount of cyanophage (PFU) released after 24 hours was considerably higher than in series 1 (cf. Fig. 3 and 6). The addition of CO₂/air in the presence of nutrients, however, resulted in a short delay in viral replication that for a short time favoured the algae (cf. Fig. 6 and 7).

These results partly agree with observations from field experiments where a combined reduction in pH and addition of nutrients (N and P) accelerated the blue-green algal collapse (Shapiro 1973). In the laboratory systems the pH was lowered by the addition of nutrient solution.

From the analysis of NH₄-N and PO₄-P concentrations following lysis of *Plectonema*, it seems that the mode and amount of CO₂/air input are important (Fig. 8). In the field the decline of blue-green algae is often accompanied by release of PO₄-P (Shapiro et al. 1975).

When cells of *Scenedesmus* were present during lysis of *Plectonema*, no NH₄-N was detected at low pH; it was most likely utilized by *Scenedesmus*. In cultures with *Anabaena* and without

additional algal species, the released $\text{NH}_4\text{-N}$ remained in solution. It could not be determined whether low pH solely, or in combination with other factors e.g. bacteria or lysing agents (Gunnison & Alexander 1975) following lysis of *Plectonema*, was the reason that *Anabaena* failed to survive. However, at high pH *Anabaena* remained viable.

These findings may be useful in experiments in natural ecosystems, e.g. artificial circulation of whole lakes, with respect to nutrient requirements and competition between blue-green algae (N_2 fixers in particular) and green algae.

Dependence on Age of Host Algal Culture

It is evident that *Plectonema* in the exponential growth phase showed the strongest "physiological resistance" to lysis (cf. Fig. 14). In the 4.5 and 6-day old culture of *Plectonema* in series 3 and 4, respectively, pH was around 11 at the time for cyanophage inoculation. The addition of CO_2 /air to the cultures stimulated cyanophage replication in both series. However, the resistance of the algae to lysis due to culture age was more pronounced than that due to the varying pH adjustments in series 4 (cf. Fig. 10 and 14). In quasi-continuous cultures Cowlshaw & Morsa (1975) found that up to 0.3 % of the algal cells survived the first round of lysis and suggested that this could not be due solely to mutation. Safferman & Morris (1964) reported that seven days were necessary to achieve lysis in a four-day culture of *Plectonema*.

Higher resistance to viral attack during the exponential phase

of growth can be compared to the usual pattern of development of blue-green algae. In Lake Erken (Sweden) *Aphanizomenon flos-aquae* steadily increased during summer before a viral infection terminated the bloom in September (Granhall 1972). Physical and chemical environmental factors (no pH values reported) varied little during the critical period; however, the abundance of *Aphanizomenon* was extremely low the following summer.

CONCLUSIONS

Although several interactions in the cyanophage - alga system studied paralleled observed events in field experiments with similar external stresses and variations, the data are far from sufficient to allow definite conclusions. On the other hand, the results are encouraging, and more experiments along similar lines should be conducted. One of the difficulties with the work reported here is that, because of time restrictions, it was not possible to repeat each experiment enough to establish strict relationships.

For example, if the results of series 1 could be validated by sufficient repetition, then the effects of small environmental changes could be more readily evaluated. In this manner it might be possible to determine, for example, whether artificial circulation of a lake, with subsequent lowering of pH and elevation of nutrient concentrations, would be more likely to cause a shift from blue-green algae to green algae at one time in the history of an algal bloom than at an earlier or

later point in that history. Such information would be of immense practical value and would be a step toward an ecological approach to lake restoration (Shapiro 1978).

The use of cyanophages is an attractive method for biological control, in that it is catalytic and avoids poisoning the general environment. The strong specificity of the cyanophages could provide selective control of blue-green algae, although development of resistant algal clones might be expected after large viral infections.

The need to understand the cause of algal blooms - both biologically and chemically - remains (Walsby 1970, Reynolds & Walsby 1975).

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Table 1. Composition and concentration of nutrient solution[★]

Compound	mg/l	mM
KCl	34	0.46
Na ₂ SiO ₃ ·9H ₂ O	58	0.20
Na ₂ CO ₃ ·10H ₂ O	54	0.19
MgSO ₄	75	0.62
CaCl ₂	36	0.48
NaNO ₃ ¹⁾	496	5.8
KH ₂ PO ₄ ¹⁾	4.7	0.032
FeCl ₃ ·6H ₂ O	2.8	0.01
EDTA	3.7	
Trace solution (Hoagland's A-Z)		

[★] modified from Hughes, Gorham and Zehnder (1958)

¹⁾ concentrations modified in some experiments

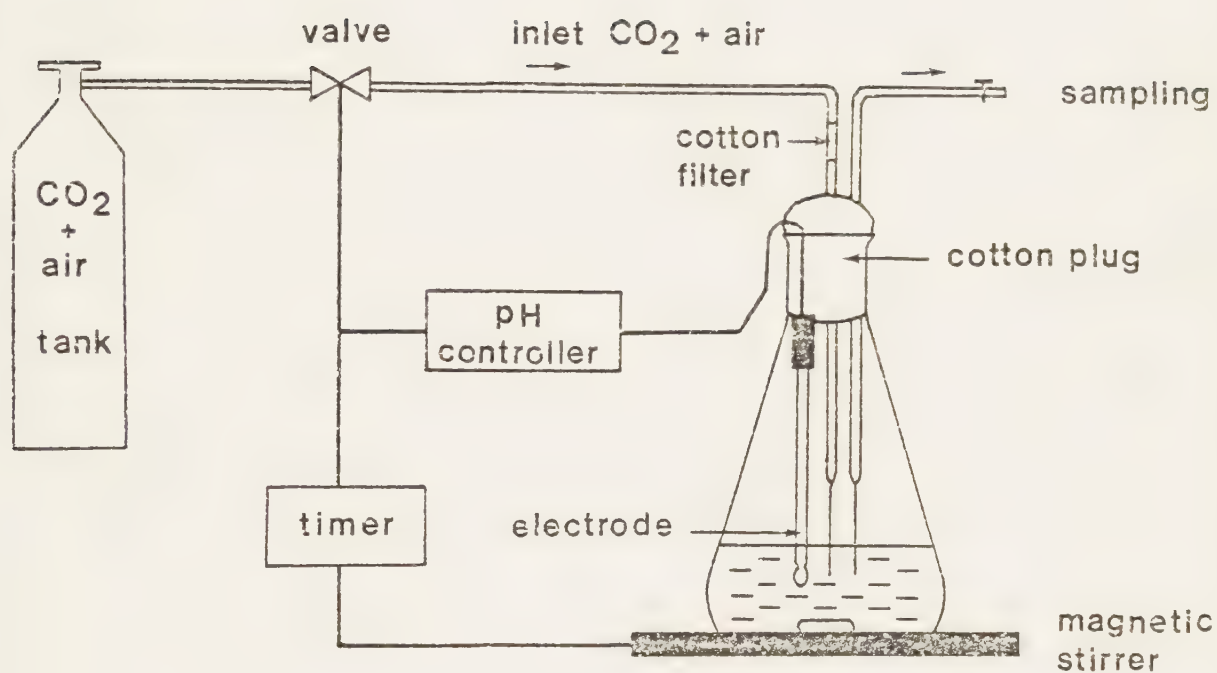


Fig. 1. Schematic diagram of one experimental unit.

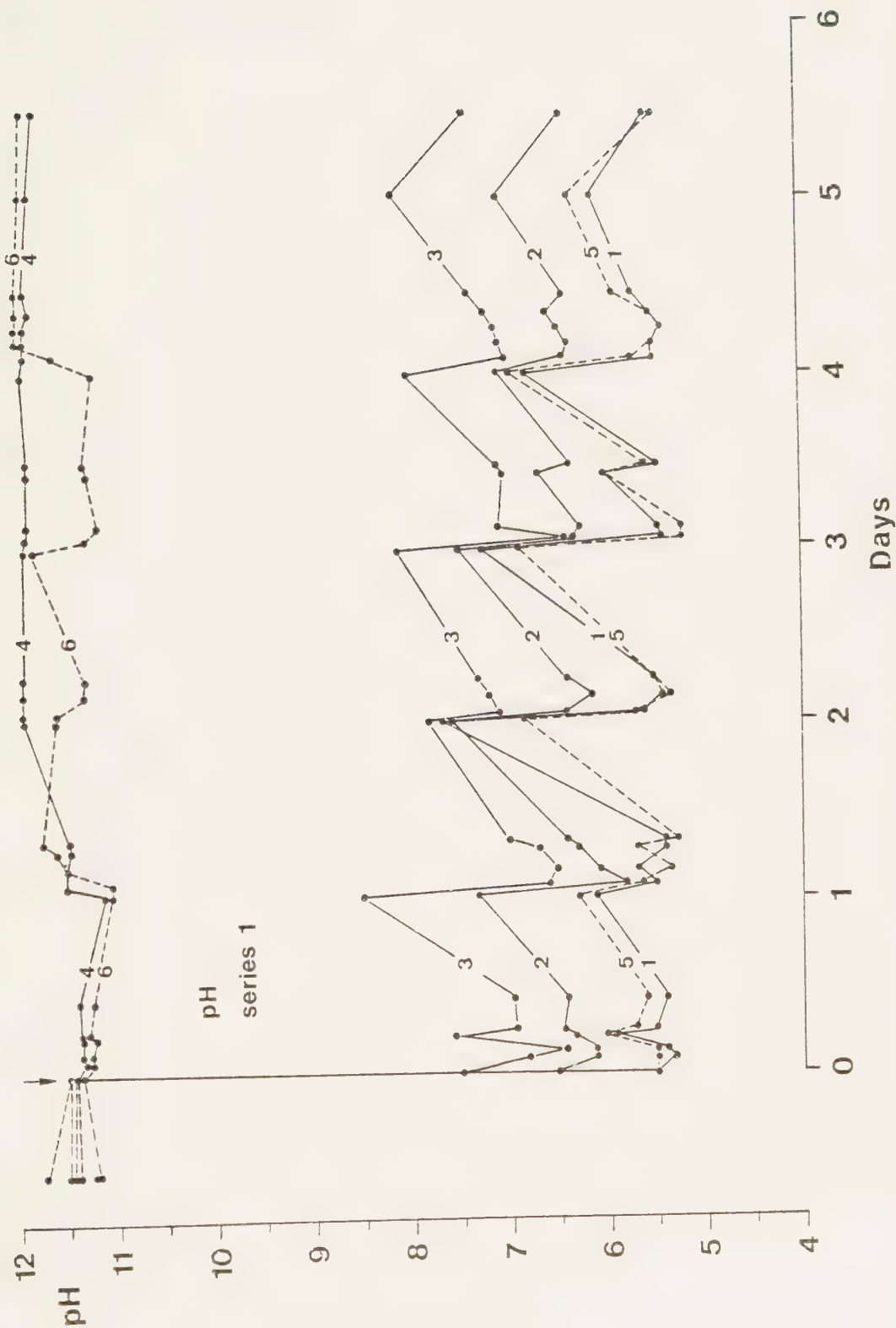


Fig. 2. pH in cultures of *Plectonema* infected with cyanophage LPP-1 (—, 1-4) and cyanophage-free (---, 5 and 6). Arrow (day 0) indicates inoculation with cyanophage and start of CO₂/air addition (1-3 and 5) to 11-day old cultures.

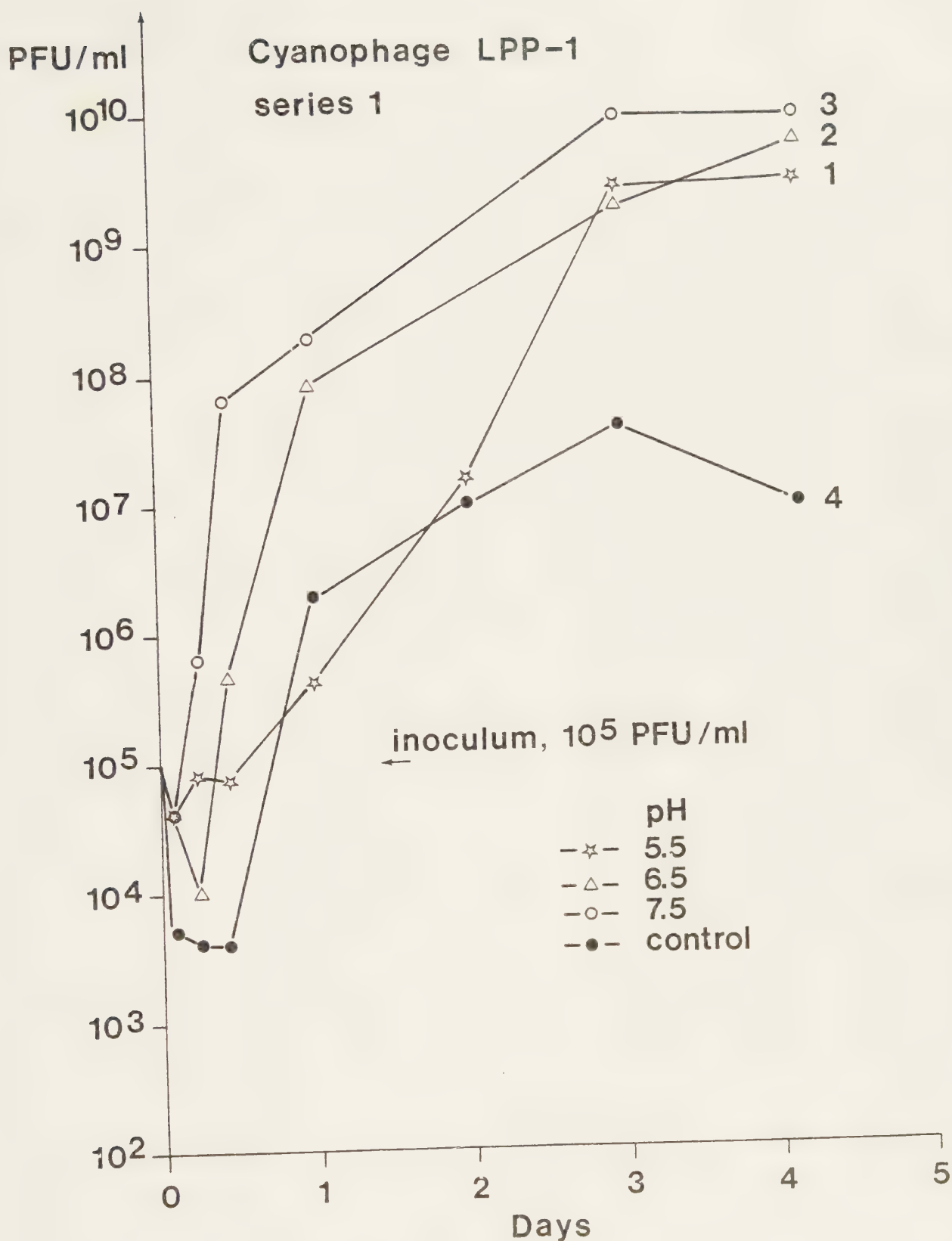


Fig. 3. Replication of cyanophage LPP-1 after inoculation on day 0 in 11-day old cultures of *Plectonema*. Control (●), pH adjusted (☆, △, ○). Culture numbers (1-4) indicated.

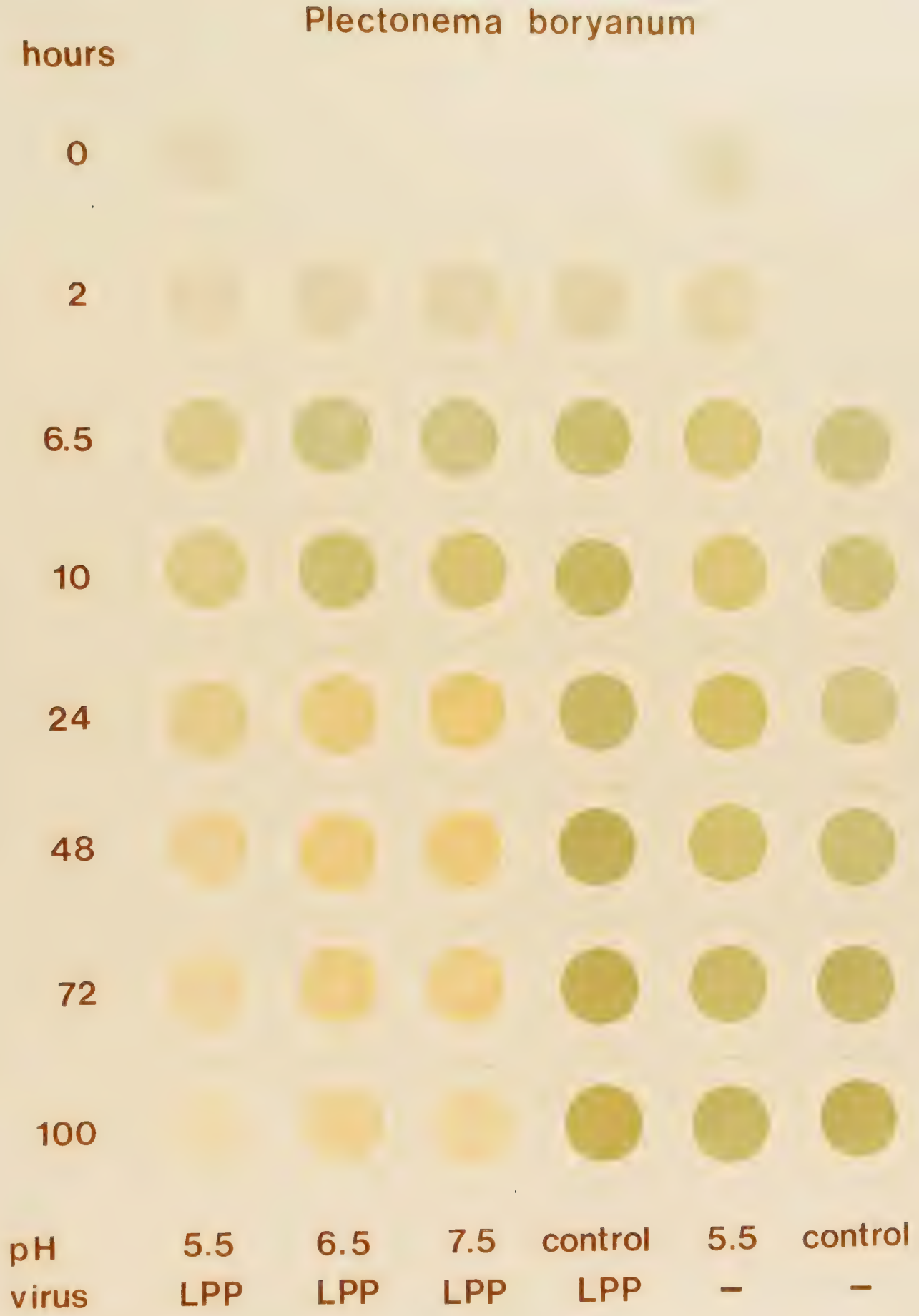


Fig 4. Appearance of *Plectonema* cultures (on 0.22 μ m Millipore membrane filters) growing under controlled and drifting pH conditions with and without inoculated cyanophages (LPP). Hour 0 indicates cyanophage inoculation and addition of CO₂/air to varying pH in the 11 days old cultures (Series 1).

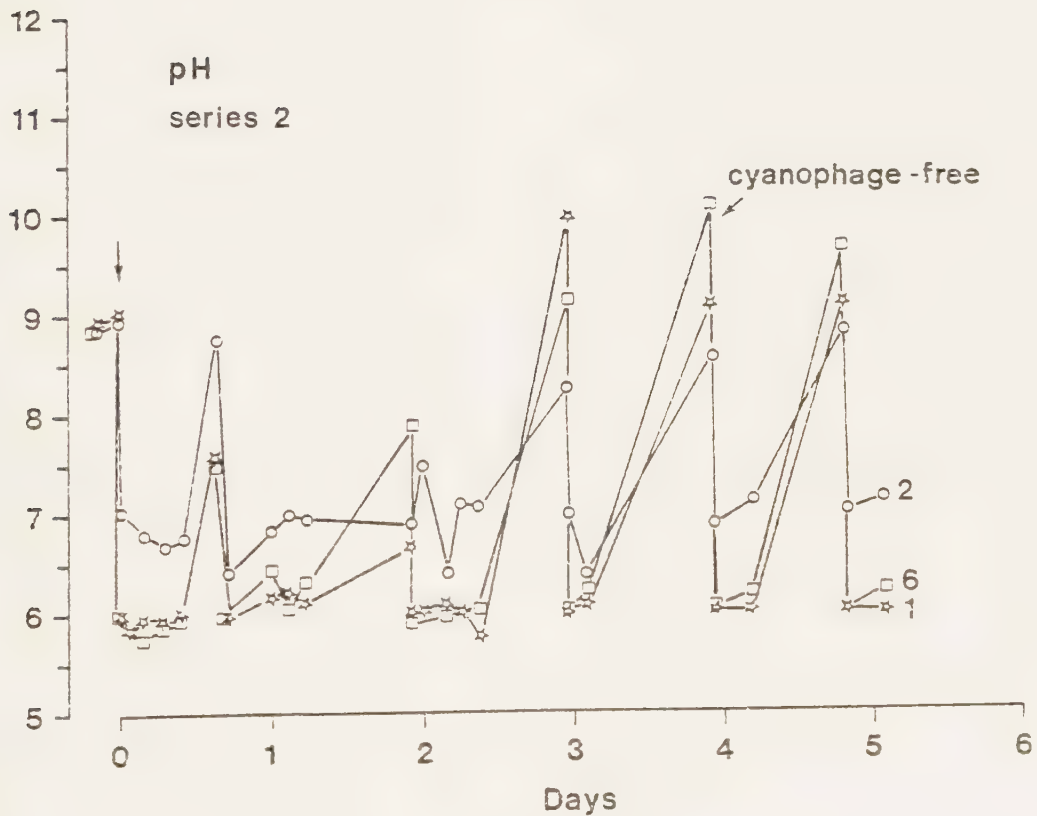
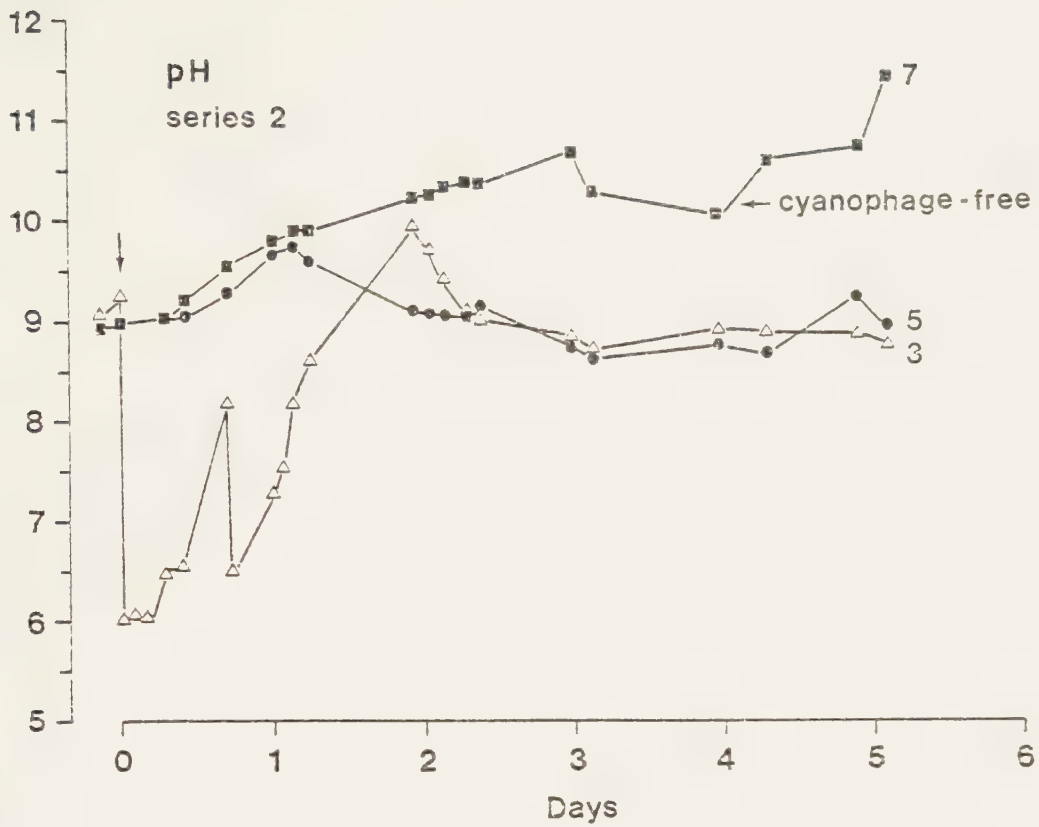


Fig. 5. pH in cultures of *Plectonema* infected with cyanophage LPP-1 (1-3 and 5) and cyanophage-free (6 and 7). Arrow (day 0) indicates inoculation with cyanophage and start of CO_2 /air addition (1-3 and 6). Culture numbers are indicated.

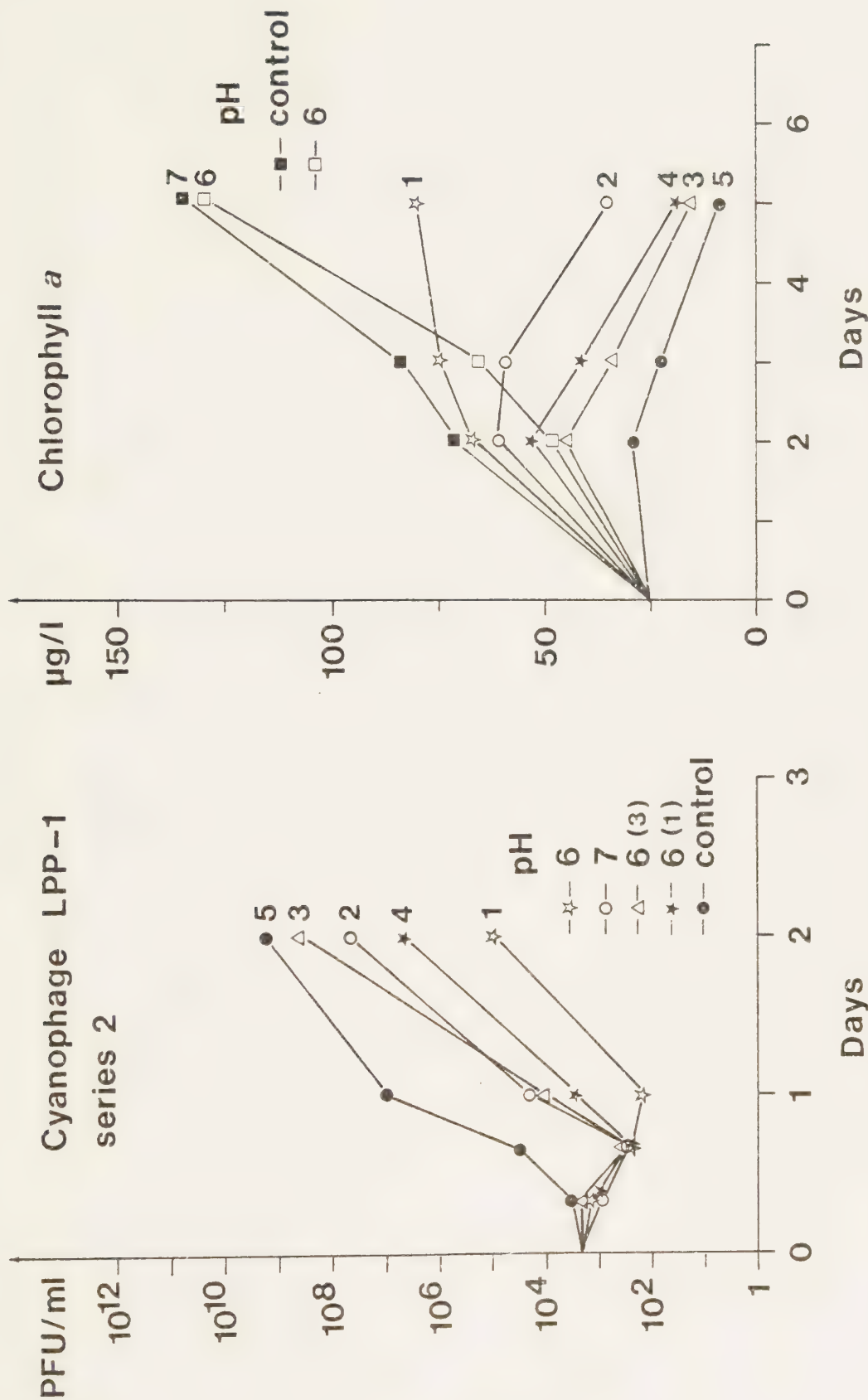


Fig. 6. (left). Replication of cyanophage LPP-1 in *Plectonema* cultures after inoculation on day 0. Control (●), pH adjusted (to value shown) (☆, ○, △, ★). Culture numbers (1-5) shown. (1) and (3) indicate 1 and 3 injections of CO₂/air.

Fig. 7. (right). Chlorophyll a in cultures of *Plectonema* infected with cyanophage LPP-1 (☆, ○, △, ★, ●) and cyanophage-free (□, ■). pH conditions shown here and in Fig. 6. Culture numbers (1-7) indicated.

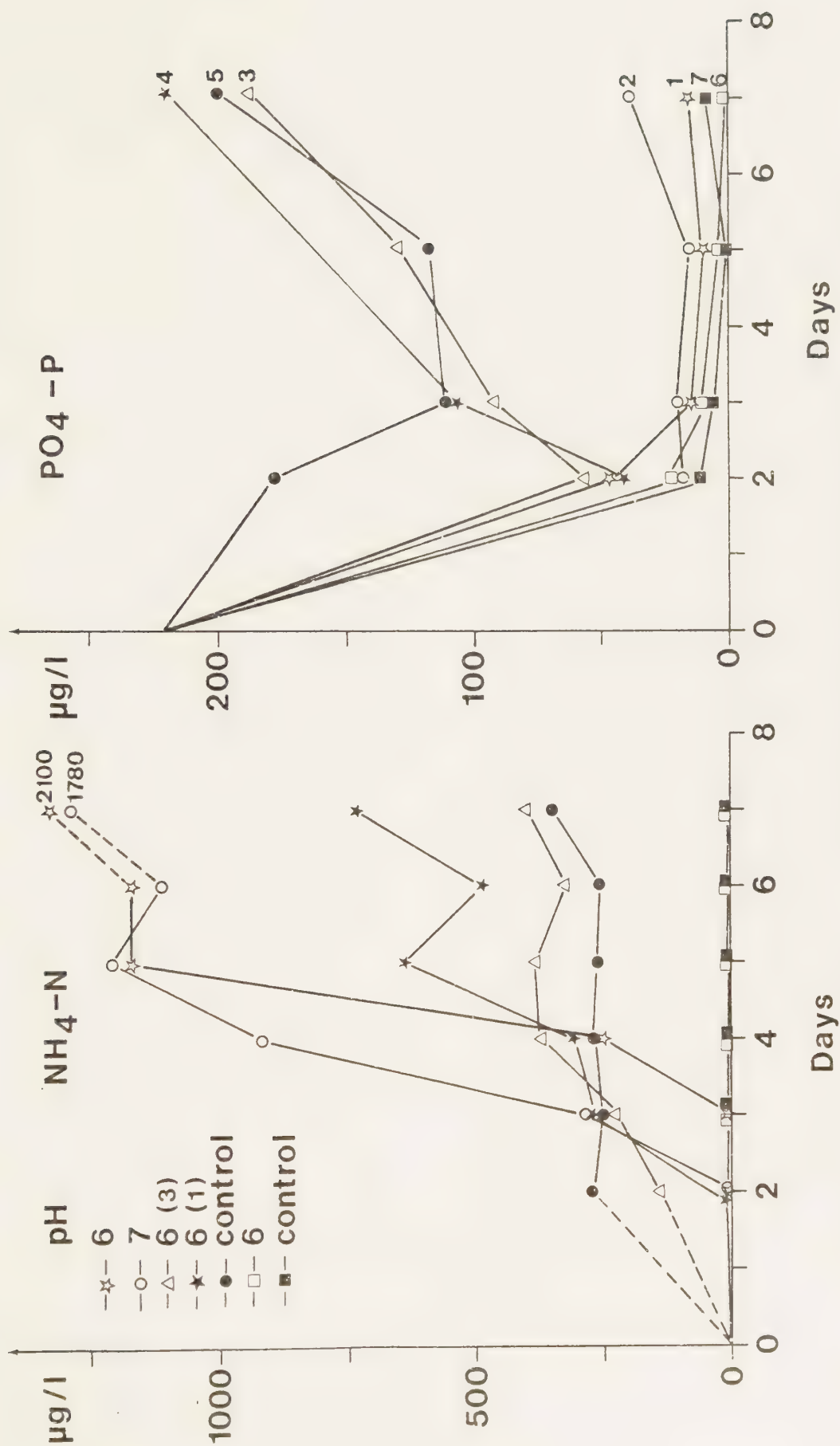


Fig. 8. Ammonia (left) and phosphate (right) in cultures of *Plectonema* infected with cyanophage LPP-1 (☆, ○, △, ★, ●) and cyanophage-free (□, ■) grown under varying pH conditions (series 2). Day 0 indicates inoculation with cyanophage and start of pH adjustment. Culture numbers (1-7) shown. (1) and (3) indicate 1 and 3 injections of CO₂/air.

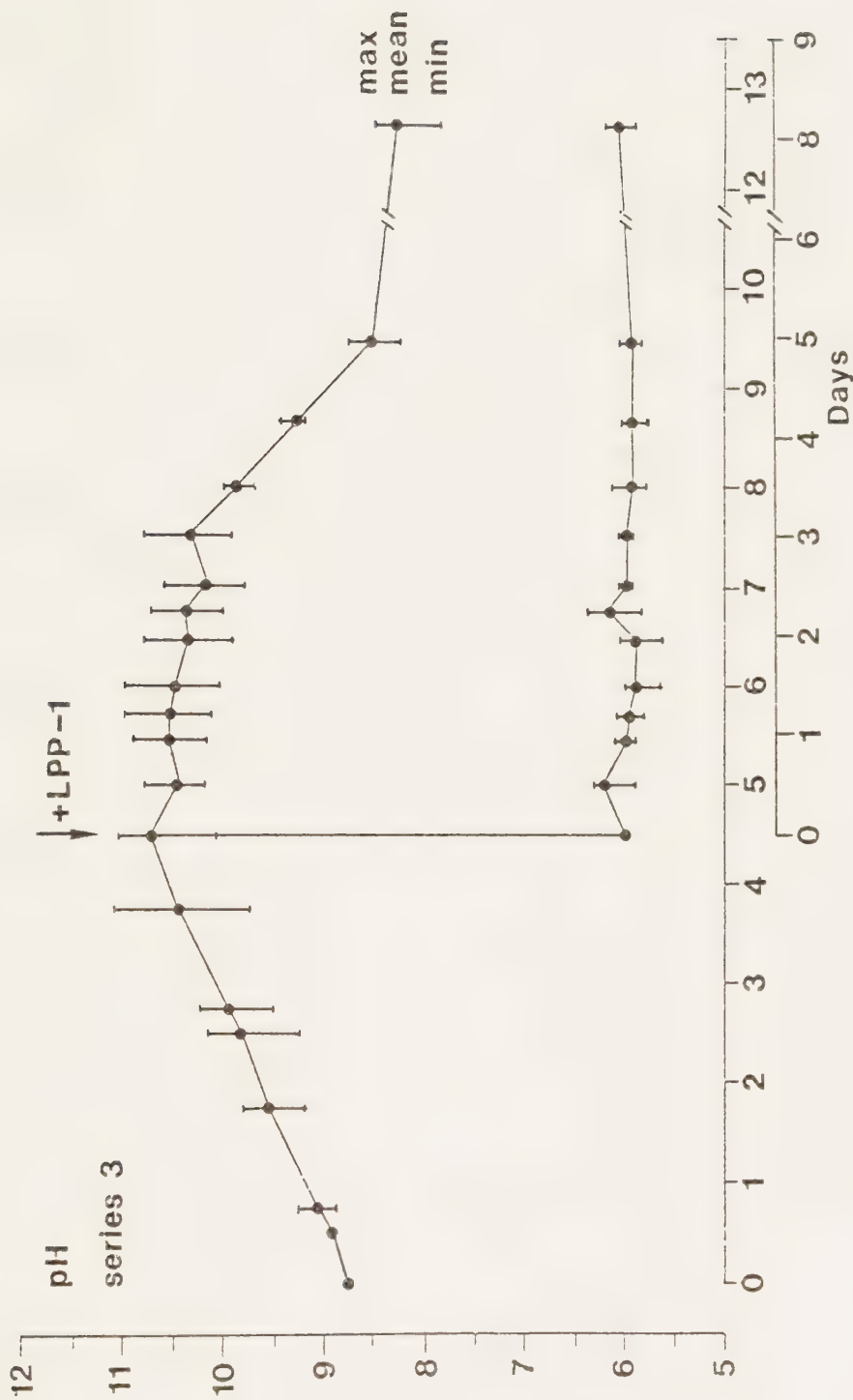


Fig. 9. pH in cultures of *Plectonema* before and after inoculation with cyanophage LPP-1 and *Scenedesmus* or *Anabaena*. Day 0 (upper scale) indicates inoculation of fresh nutrient solution with *Plectonema* cells and day 0 (lower scale) indicates start of CO₂/air addition (4 cultures) and inoculation with cyanophage (6 cultures). Dots represent mean values and vertical bars the range.

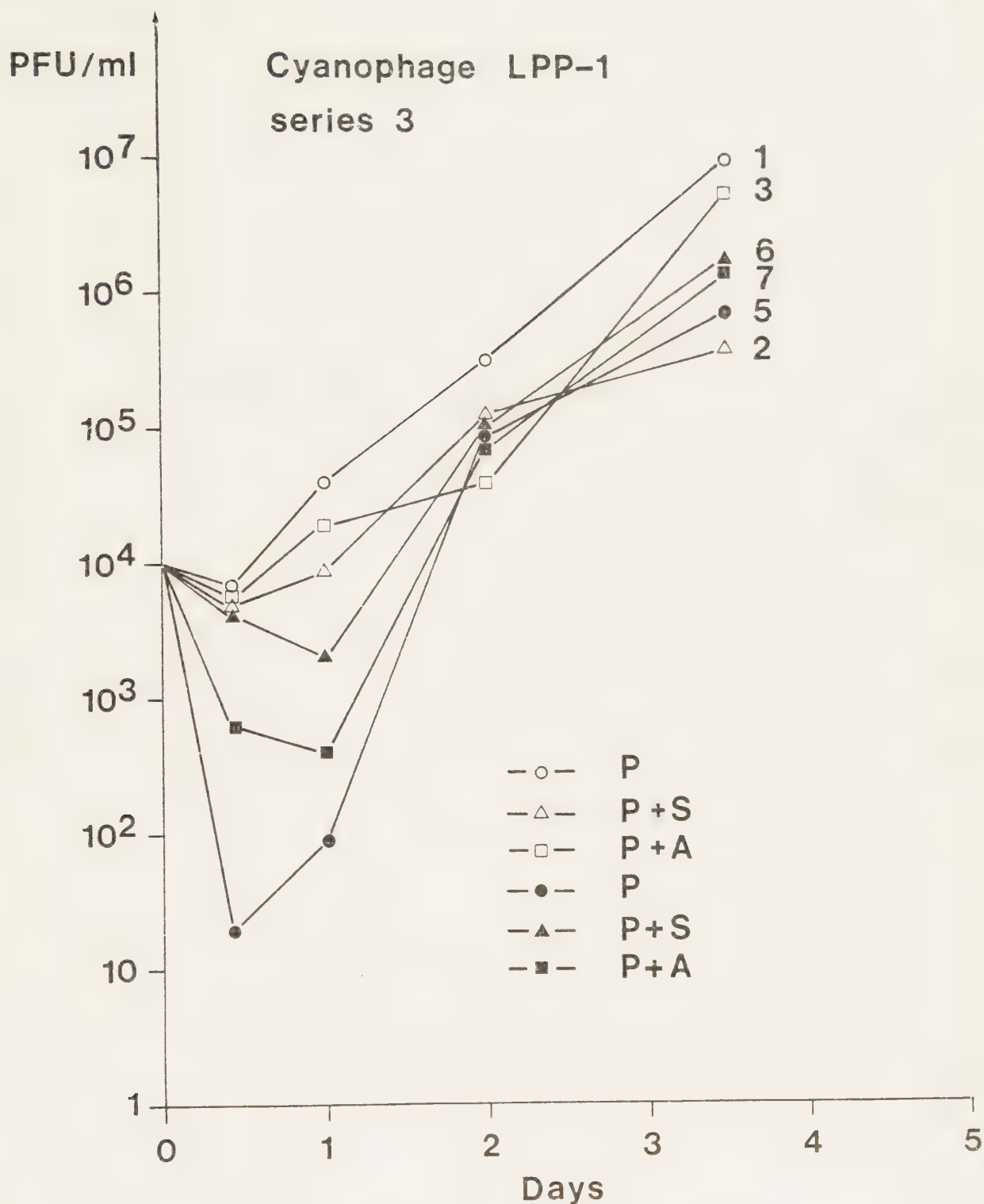


Fig. 10. Replication of cyanophage LPP-1 in algal cultures with pH adjustment (open symbols, pH ca. 6) and in controls (closed symbols). *Plectonema* (P), *Plectonema* + *Scenedesmus* (P+S) and *Plectonema* + *Anabaena* (P+A). LPP-1 inoculum was 10^4 PFU/ml. Culture numbers (1-7) indicated.

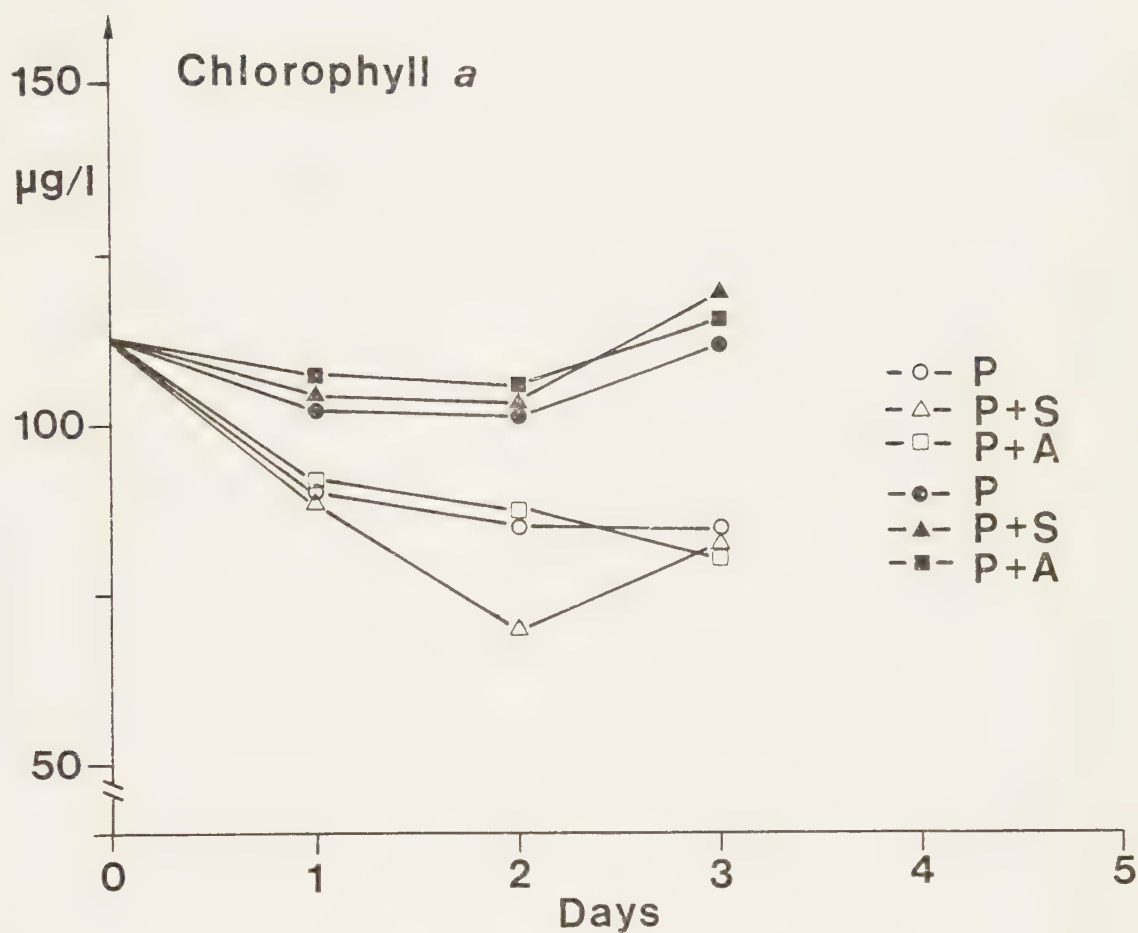


Fig. 11. Chlorophyll *a* in cultures of *Plectonema* (P), *Plectonema* + *Scenedesmus* (P+S) and *Plectonema* + *Anabaena* (P+A) infected with cyanophage LPP-1 and grown with pH adjustment (open symbols) or as controls (closed symbols). *Scenedesmus* and *Anabaena* were inoculated simultaneously with the cyanophage (day 0) in 4.5-day old *Plectonema* cultures (series 3).

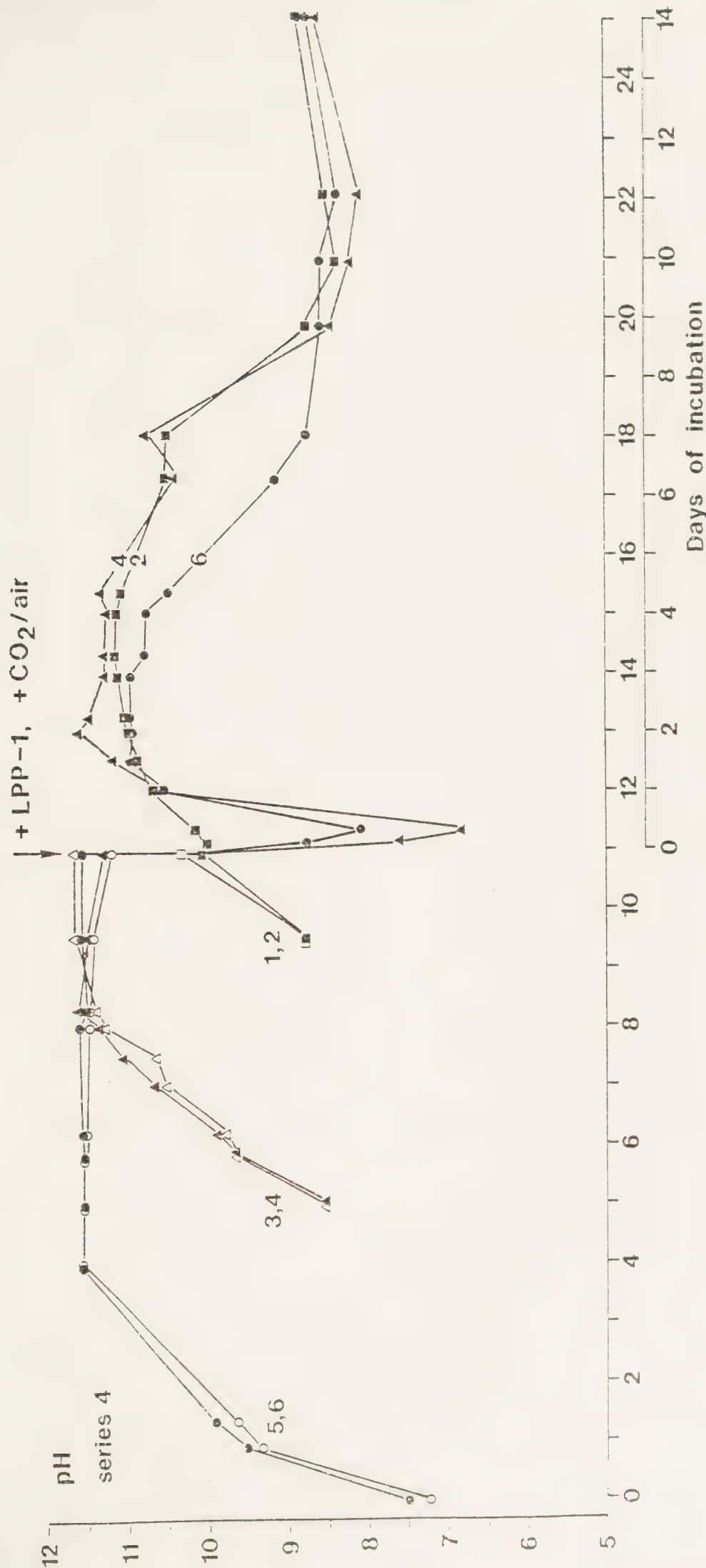


Fig. 12. pH in cultures of *Plectonema* before and after inoculation with cyanophage LPP-1 and start of CO₂/air addition (day 0, lower scale). Algae were grown for 1.5, 6 and 11 days before the cyanophage was added. Cultures with pH adjusted to ca. 7 are omitted in the diagram. Culture numbers (1-6) are indicated.

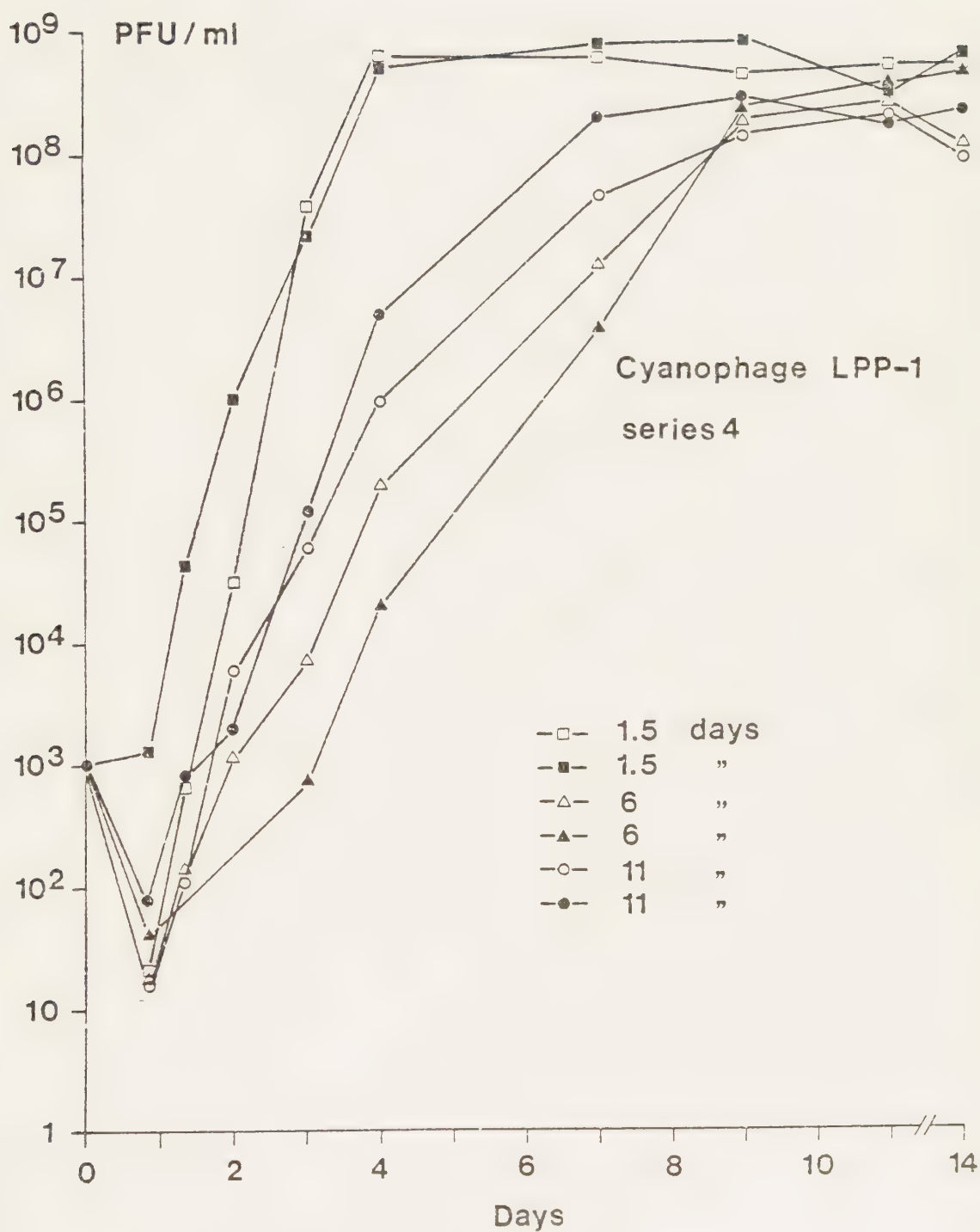


Fig. 13. Replication of cyanophage LPP-1 in cultures of *Plectonema* grown for 1.5, 6 and 11 days before inoculation with the cyanophage. Open symbols indicate cultures with frequent addition of CO₂/air (pH ca. 7), and closed symbols indicate no (■) and one (●, ▲) injection of CO₂/air.

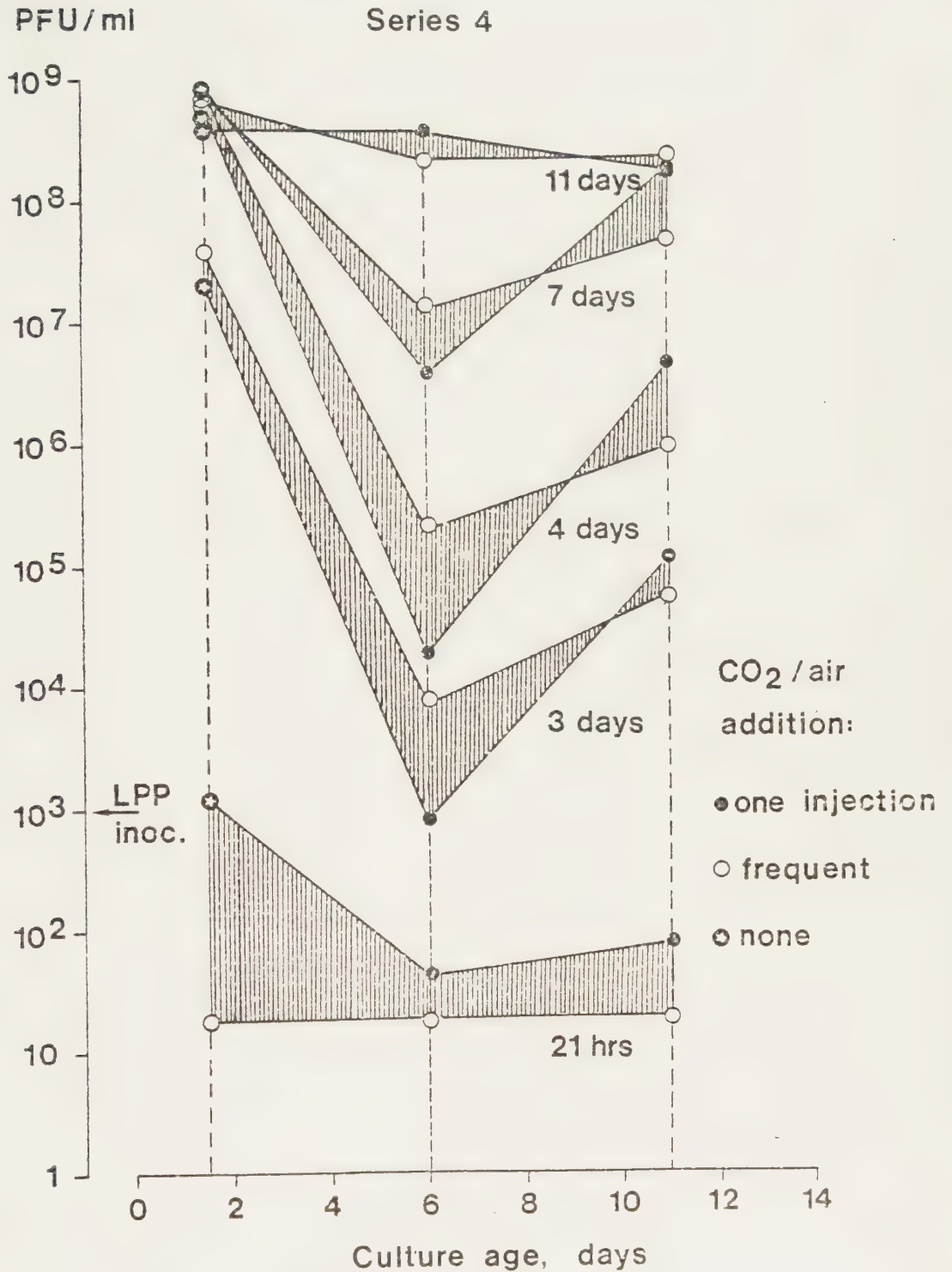


Fig. 14. Concentration of cyanophage LPP-1 in relation to *Plectonema* culture host age and time following cyanophage inoculation. Shaded areas indicate difference between cultures receiving no, one and frequent injections of CO₂/air.



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PHOSPHORUS AS A GROWTH-CONTROLLING FACTOR
FOR PHYTOPLANKTON IN LAGO PARANOÁ, BRASILIA

Gunilla Lindmark

INSTITUTE OF LIMNOLOGY
University of Lund

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ABSTRACT

Nutrient bioassays were performed under both laboratory and *in situ* (plastic bags) conditions. Combined with data from a monitoring study, the results have been analysed as to causes and consequences of variations in nutrient input to Lago Paranoá, Brasilia.

A close linkage between phosphorus and chlorophyll a was demonstrated both in the lake and in the experiments. Variations in plant nutrients within the lake strongly influenced the cellular phosphorus content of the phytoplankton, a monoculture (>99 %) of *Anabaenopsis raciborskii*. Phosphorus content in phytoplankton varied between 0.4-0.08 % of algal fresh weight, and particulate phosphorus to chlorophyll a ratios (by weight) varied between 1:1.5 and 1:3.5. Inorganic nitrogen was mostly in excess in the open lake water, but if necessary the *Anabaenopsis* population was able to fix atmospheric nitrogen. Green algae are continuously inoculated to the lake via a tributary, but they are most probably outcompeted by the blue-green algae at the low N:P ratios maintained in this area by localized sewage discharge.

Phosphorus control is the only way to retard algal growth in Lago Paranoá, reverse the eutrophication and regain the recreational value of the lake.

PHOSPHORUS AS A GROWTH-CONTROLLING FACTOR FOR PHYTOPLANKTON IN LAGO PARANOÁ, BRASILIA

By Gunilla Lindmark

Institute of Limnology, University of Lund,
Lund, Sweden

INTRODUCTION

Increased algal development and nuisance blooms are well-known consequences of the cultural eutrophication of lakes (Reynolds & Walsby 1975). Eutrophication management based primarily on phosphorus control is sometimes considered too simple, although there is now a general agreement that phytoplankton will be phosphorus-limited in a majority of lakes. Investigations in lakes and enclosures have demonstrated the existence of mechanisms capable of compensating deficiencies of carbon and nitrogen in algae by uptake from the atmosphere (cf. Talling 1976, Schindler 1977, Lean et al. 1978), but phosphorus forms no gaseous compound of biological significance. The availability and abundance of biologically-active forms of phosphorus in lakes are reviewed by Reynolds (1978).

Analysis of global data on fresh water phytoplankton production, chlorophyll and various nutrient parameters has shown that nutrient input, after correction for water renewal time, plays the preeminent role in controlling phytoplankton production and biomass (Schindler 1978). However, there also exists

some evidence for correlation between latitude and production (Brylinsky & Mann 1973).

In tropical waters the growing season is never interrupted, as temperature and light usually are above the levels at which algal growth is limited. Thus, nutrient availability probably plays a major role in controlling even the seasonal pattern of algal growth. Therefore, external nutrient input could cause severe damage to tropical ecosystems and, depending on water renewal time, result in negative long-term changes in water quality.

In the man-made Lago Paranoá in Brasilia (Brazil) a continuous flow of nutrients enters the lake from treatment plants and polluted tributaries, and water quality has rapidly deteriorated. This paper presents an experimental study on the lake, by means of nutrient bioassays, to identify the triggering factor for increased algal growth and to clarify the response of the phytoplankton community to changed nutrient levels.

These nutrient changes were simulated in laboratory and *in situ* experiments by mixing lake water with varying amounts of:

- a) phosphorus and/or nitrogen
- b) sewage water effluent before and after P precipitation
- c) polluted water from tributaries and oxidation ponds
- d) uncontaminated water from the headwaters of a tributary
- e) herbivorous fish; *Tilapia nilotica*

The study was an integrated part of a project designed to

evaluate the trophic status of and nutrient loading on the lake in order to design measures for restoration (Björk 1975).

The results from the bioassays are analysed together with data on primary productivity and phytoplankton phosphorus to evaluate consequences of continued or reduced nutrient loading for the future of Lago Paranoá.

BACKGROUND DATA ON THE LAKE

Lago Paranoá is a man-made lake created in 1960 to be an aesthetic and recreational resource for the capital, Brasilia (Fig. 1). Morphometric and hydrological data are presented in Table 1. The surroundings of the lake consist mainly of eroded savanna grassland (cerrado) and exposed red-brown soil. Lago Paranoá has four main tributaries and is drained by Rio Paranoá (Fig. 1). A dam is built at the outflow.

Sewage water discharges into the lake from two treatment plants (activated sludge), ETE-Sul and ETE-Norte (Fig. 1), have rapidly ruined water quality and thus reduced the recreational value of the lake. Low Secchi disc transparency and a heavy bloom of blue-green algae (>99 % dominance of *Anabaenopsis raciborskii*, Cronberg 1978) now characterize the lake (Table 2).

Before the pollution (sewage input) of Lago Paranoá, water quality resembled conditions in the uncontaminated Lago Santa Maria, a protected lake created in 1967 for drinking water supply (Table 2). Low conductivity ($12 \mu\text{S cm}^{-1}$ in Lago Santa Maria) and thus weak buffer capacity is a characteristic fea-

ture of the man-made lakes in the area.

The area experiences a rainy season from November until April and one slightly cooler dry season. Lago Paranoá circulates once a year, from mid-June through July, and then stratifies.

MATERIALS AND METHODS

Sampling

For monitoring of water quality, sampling was carried out every second week at the main stations in the lake (Fig. 1, A-E). Additional sampling was generally conducted for the experimental part of the study. Lake water was collected at station C (Fig. 1) for laboratory bioassays. *In situ* bioassays were performed in the northwestern bay close to laboratory facilities (ETE-Norte, Fig. 1). However, less than 5 % of sewage water P input (i.e. $<0.17 \text{ mg/m}^2 \cdot \text{d}$) comes from the north treatment plant, ETE-Norte (Hilmer 1978). Water quality in the bay was monitored every second to third day during the *in situ* experiments.

Uncontaminated water was collected from Rio Fundo headwaters (Fig. 1) for dilution experiments. To increase nutrient concentrations, sewage water effluent from ETE-Sul (ca. 55 % of total P input to the lake, Hilmer 1978), polluted water from Rio Fundo (Fundo 2, Fig. 1) and effluent water from the Guará oxidation pond that flows via Rio Fundo to the southwestern bay of Lago Paranoá were used.

The sewage water effluent from ETE-Sul used in the small-bag experiment in 1977 (Table 4, series 2) was mixed from subsamples collected every second hour during the day (0900-1700) to obtain representative nutrient concentrations.

Sewage effluent was also treated with $\text{Al}_2(\text{SO}_4)_3$ in the laboratory to precipitate phosphorus. For comparison with results from bag experiments with sewage water enrichment, a transect in the southwestern bay (Rio Fundo inlet - station A) was sampled.

Tilapia nilotica was collected from fertilized fishponds, and the fishes were starved in laboratory aquariums before being stocked in the plastic bags.

Methods for Laboratory and *In Situ* Bioassays

Laboratory bioassays were performed according to the batch culture technique. Water samples were either filtered (Whatman GF/C) and inoculated with a unialgal culture of *Microcystis aeruginosa* (Wis 1036) or *Scenedesmus* sp. (Rhee 1972), or incubated with the natural algal community.

250 ml of the algal suspensions, in 0.5-liter flat bottom round flasks, were incubated under constant light (ca. 3000 lux) and $23 \pm 3^\circ\text{C}$. Various combinations of nutrients were added, and the algal response was followed by chlorophyll a and fresh weight measurements.

As the natural algal community in Lago Paranoá was almost a monoculture of *Anabaenopsis raciborskii* (Cronberg 1978), the

use of the natural algal community in the assays was more attractive than the use of laboratory cultures.

The *in situ* bioassays were carried out in series of transparent plastic bags (polyethylene) suspended from rafts in the lake (Shapiro et al. 1975). Two sizes of bags were used; one with a surface of 0.2 m^2 and volume 145 liter, and the other with a surface of 1.0 m^2 and volume 1400 liter. The bags were sampled about every second day for physical, chemical and biological analyses.

Analytical Methods

Physical and chemical analyses

pH was measured electrometrically with a combined glass-calomel electrode.

Total P was digested to $\text{PO}_4\text{-P}$ by $\text{K}_2\text{S}_2\text{O}_8$ and analysed as soluble reactive phosphorus according to Murphy & Riley (1962).

$\text{NH}_4\text{-N}$ was determined according to Chaney & Marbach (1962),

$\text{NO}_2\text{-N}$ according to Bendschneider & Robinson (1952), and

$\text{NO}_3\text{-N}$ with the phenoldisulphonic method (Standard methods 1965). Particulate phosphorus was calculated as the difference between total and dissolved (Whatman GF/C filtration) phosphorus. Suspended solids (particulate matter) were analysed on prewashed glass fiber filters (Whatman GF/C), dried over night (105°C).

Chlorophyll a

Appropriate volumes of algal suspensions and lake water were

filtered under vacuum on 5.5 cm glass fiber filters (Whatman GF/C) and washed with a small volume of distilled water. The filters were ground in 90 % acetone, and chlorophyll was extracted for 12 hours in the dark at 4-6°C. Chlorophyll a was estimated according to Lorenzen (1967).

Primary productivity and respiration

Photosynthetic and respiratory activities in the lake water were measured by suspending integrated (0-0.5 m and 0.2-2 m) water samples in light and dark bottles at various depths. The water samples were exposed for three hours (1100-1400) and then analysed for dissolved oxygen (Winkler method).

For practical reasons the samples, collected at different stations, were all exposed at station E (Fig. 1). In this way an identical light and temperature climate was obtained for all samples during the exposure.

Experimental Design

Two laboratory experimental series were performed (Table 3) to aid in the design of subsequent *in situ* plastic bag experiments. Laboratory assays with *Microcystis* were also performed on lake water from a transect in the southwestern bay (Fundo Bay - station A) to evaluate the availability of dissolved nutrients.

Three experimental series were run *in situ* in plastic bags (Table 4). One series was run in October, 1976, and the other two in November, 1977.

The sewage water added in series 1 contained 3.3 mg $\text{PO}_4\text{-P/l}$ and 18.5 mg $\text{NH}_4\text{-N/l}$, and the water used from Fundo 2 contained 1.5 mg $\text{PO}_4\text{-P/l}$ and 8.7 mg $\text{NH}_4\text{-N/l}$.

In series 2 the sewage effluent contained 2.5 mg $\text{PO}_4\text{-P/l}$ and 20 mg $\text{NH}_4\text{-N/l}$, and the effluent from Guarã oxidation pond 1.5 mg $\text{PO}_4\text{-P/l}$ and 12 mg $\text{NH}_4\text{-N/l}$. The water from Guarã pond also contained large amounts of green algae (mostly *Micractinium*, 600 $\mu\text{g Chl } a/\text{l}$) and zooplankton (rotifers, ca. $10^6/\text{l}$).

The sewage water used in the large bags (series 3) contained only 0.52 mg $\text{PO}_4\text{-P/l}$. (It was collected early on a Monday morning.) The concentration of $\text{PO}_4\text{-P}$ in the water from Guarã used in series 3 was twice that used in series 2.

Based on 9 dial investigations, the average total P concentration in the sewage effluent from ETE-Sul is 2.8 mg P/l (Hilmer 1978).

Solutions of NH_4Cl and KH_4PO_4 were used as N and P sources. $\text{NH}_4\text{-N}$ was added to the bags to give an increase of 1 mg N/l, and $\text{PO}_4\text{-P}$ was increased with 0.1 mg P/l.

RESULTS

Algal Response to Nutrient Changes in Laboratory Assays

Preliminary algal assays showed that *Microcystis* grew well in filtered lake water enriched with $\text{PO}_4\text{-P}$ (Table 5). Addition of both phosphorus and nitrogen further increased the chlorophyll *a* concentration. *Scenedesmus* also grew well when nitrogen and phosphorus were supplied (Table 5). This indicated that inorganic N in the Lago Paranoá water was depleted

and that nitrogen had become the secondary growth-limiting element.

The natural algal community (99 % dominance of *Anabaenopsis*, Cronberg 1978) collapsed when fresh samples were incubated in the laboratory without nutrients or when only $\text{PO}_4\text{-P}$ was added. When phosphorus and nitrogen were added together, the chlorophyll a concentration increased, however (Table 5).

When fresh samples of Lago Paranoá water were diluted with uncontaminated water from the headwaters of the tributary Rio Fundo (Table 3, series 2), the algal trichomes became thinner, fell apart and declined in numbers. By the 6th day of incubation the algal community had almost collapsed (Table 6). If phosphorus and nitrogen were added in combination with the nutrient-poor water, the algae remained normal and the biomass increased (Table 6).

When water samples from Lago Paranoá were enriched with polluted water from Rio Fundo (Table 3, series 2; Fundo 2 cf. Fig. 1), algal biomass increased after a 5 % addition (Table 6). This addition increased the inorganic phosphorus concentration by $110 \mu\text{g PO}_4\text{-P/l}$ and nitrogen by $500 \mu\text{g NH}_4\text{-N/l}$. Larger additions of Fundo 2 water (10 and 25 %) did not particularly stimulate the *Anabaenopsis* population, and biomass remained almost the same or decreased (Table 6). The addition of Fundo 2 water also involved addition of *Micractinium* cells, the dominating alga in Fundo 2. However, *Micractinium* numbers had declined strongly after 6 days of incubation. When a filtered

mixture of Lago Paranoá and Fundo 2 water was inoculated with a *Microcystis* or a *Scenedesmus* culture (Table 3), the *Microcystis* cells grew well while *Scenedesmus* did not. In filtered 100 % Fundo 2 water *Scenedesmus* grew well, however. To investigate these preliminary indications of nutrient limitation, *in situ* experiments were performed.

Algal Response to Nutrient Changes in *In Situ* Experiments

The main purpose of the *in situ* experiments was to investigate the response of the natural algal community *in situ* to nutrient changes and to phosphorus concentrations in particular (Table 4).

The essential findings of the first *in situ* bioassay (series 1) were:

- 1) In all bags where $\text{PO}_4\text{-P}$ was supplied (Table 4, bags 5, 6 and 7), it was immediately taken up by the algae. After 18 hours less than $3\ \mu\text{g}\ \text{PO}_4\text{-P/l}$ and $15\ \mu\text{g/l}$ total dissolved P was recovered. During the next 8 days less than $10\ \mu\text{g/l}$ total dissolved P was found in all bags. In bag 6 (5 % sewage addition) $\text{PO}_4\text{-P}$ ($30\ \mu\text{g}\ \text{P/l}$) had been released by the 11th day of the experiment.
- 2) The uptake rate of ammonia was slower (Fig. 2). In bag 8, where $\text{PO}_4\text{-P}$ had been removed from the sewage water (cf. Table 4), ammonia was detectable until the 8th day of the experiment.
- 3) pH and turbidity increased rapidly as a result of algal metabolism and increase in biomass (Fig. 2 for pH; turbi-

dity > 30 JTU).

- 4) Chlorophyll a concentrations increased about 3-fold with additions of sewage effluent and Fundo 2 water (Fig. 3). The sewage effluent without phosphorus (P precipitated with $\text{Al}_2(\text{SO}_4)_3$) had only a minor effect on the chlorophyll a concentration, however.
- 5) The presence of fish (*Tilapia nilotica*) in bag 2 (Table 4) did not affect either chlorophyll a or algal biomass until the end of the experiment (Fig. 3, Table 9). Although the fish were starved before being stocked in the bag, the presence of fish increased the phosphorus concentration of the water (Table 9). Neither fish nor algae appeared healthy by the end of the experiment.
- 6) Dilution of lake water by uncontaminated water resulted only in the algal filaments' becoming thinner. No collapse occurred. Chlorophyll a decreased only according to the dilution factor. Apparently *Anabaenopsis* could tolerate the low nutrient concentrations.

By the 14th day of the experiment the algal community had collapsed in bags with sewage and Fundo 2 water (Fig. 3). In 3 days chlorophyll a dropped from 350 to 60 $\mu\text{g}/\text{l}$ in the bag with 5 % sewage water, large amounts of phosphate (50 $\mu\text{g PO}_4\text{-P}/\text{l}$) and ammonia (Fig. 2) were released, and a community of ciliates developed.

The addition of sewage water to the bags also supplied large quantities of inorganic nitrogen compounds. The main objective

of series 2 (Table 4) was to investigate whether the natural algal populations would fix atmospheric nitrogen - making phosphorus the element controlling algal growth in Lago Paranoá - or if nitrogen also should be considered a limiting nutrient as was shown in the laboratory assays with *Microcystis*, a non N_2 -fixing algal species.

Consequences of algal species competition were also investigated. In Fundo Bay green algae enter from Rio Fundo, but they disappear in favour of blue-green algae further into the lake. Water from Guarã oxidation pond was used to take advantage of the high plankton concentration and thereby increase the initial plankton inoculum in the bag. A 5 % addition involved a contribution of $5 \cdot 10^4$ zooplankters (rotifers) and 30 μg chlorophyll a (*Microactinium* cells) per litre mixture.

Phosphorus and ammonia uptake, temperature, pH, transparency and chlorophyll a concentration in series 2 are presented in Fig. 4-8.

The essential findings were:

- 1) Sewage water additions resulted in an almost identical response by the algal community to that in series 1 regarding P and N uptake (Fig. 6 and 7) and increase in chlorophyll a concentrations (cf. Fig. 3 and 8).
- 2) PO_4 -P and NH_4 -N supplied together raised chlorophyll a in accordance with the amount of P added and in a manner similar to the situation when sewage effluent was added (Fig. 8).

- 3) When only $\text{PO}_4\text{-P}$ was supplied, P uptake was fast (Fig. 6) while chlorophyll a increased slowly until the 7th day due to lack of nitrogen (Fig. 8). During the next 3 days, however, chlorophyll a reached a concentration almost the same as when $\text{NH}_4\text{-N}$ was supplied in combination with $\text{PO}_4\text{-P}$ (Fig. 8). On the 16th day of the experiment the algae were still actively growing. Heterocysts had developed on the algal trichomes after 3 days (Cronberg 1978), and nitrogen could thus be fixed from the atmosphere.
- 4) Addition of only $\text{NH}_4\text{-N}$ did not stimulate the algae, and chlorophyll a did not differ from concentrations in the control bags (Fig. 8). The N uptake rate and algal response were similar to results when sewage without P (Table 4, series 1) was supplied (cf. Fig. 2 and 7).
- 5) Addition of water from Guarã oxidation pond in bag 6 caused a very rapid increase in chlorophyll a, more than 3-fold in 2 days, but by the 7th day the algal community had collapsed, and transparency was greater than the depth of the bag (Fig. 8). However, no $\text{PO}_4\text{-P}$ or inorganic N were released during the 16 days of the experiment. The dissolved organic phosphorus concentration remained approximately the same throughout the experiment (Fig. 6).

In series 3 only two bags were saved from damage; the control and the bag with sewage water addition (Table 4). Results from these bags showed that even small additions of phosphorus, $13\ \mu\text{g}\ \text{PO}_4\text{-P/l}$, were immediately reflected in the chlorophyll a concentration. On this occasion $40\ \mu\text{g}\ \text{Chl}\ \underline{\text{a}}/\text{l}$ was produced.

Variations in Water Quality within Lago Paranoá and Phytoplankton Phosphorus

Variations in water quality in the lake were directly related to pollution sources and input of phosphorus and nitrogen. A variation in phosphorus concentration was immediately reflected in a change in chlorophyll a concentration (Fig. 9). Furthermore, the linkage between phosphorus and chlorophyll a was independent of the ammonia concentration.

The relationship between chlorophyll a and total phosphorus (Fig. 10) was very significant at sampling stations not directly affected by sewage discharge or polluted tributaries, i.e. stations C and D (Fig. 1 and 10). Total P concentrations were usually below 50 $\mu\text{g P/l}$ at these stations. A strong correlation was found between the two parameters at station C, $r = 0.94$ for $n = 9$ (Fig. 10), and the chlorophyll a yield per unit phosphorus was high, 3.5 $\mu\text{g Chl a per } \mu\text{g P}$. The ratio of total N to total P (by weight) was >40 (Enell 1978), and the inorganic N present was mostly ammonia.

At sampling stations located closer to the treatment plants and polluted tributaries (stations A, B and E; Fig. 1), the water was constantly affected by fluctuations in nutrient input. The algae at these stations usually contained more phosphorus per unit biomass (chlorophyll a and fresh weight) than did the algae at the main station C (Fig. 10 and Table 7). The dissolved phosphorus fraction was usually less than 20 % of total P. This might indicate that uptake of phosphorus by the algae generally keeps pace with the input of phosphorus

to the lake water.

Total phosphorus often exceeded 100 $\mu\text{g P/l}$ at station A, the most polluted of the main stations (Fig. 10). Chlorophyll a to total phosphorus ratios varied between 2:1 and 1:1, and the ratios between total N and total P fluctuated between 15:1 and 30:1 depending on prevailing P concentrations. The algae were bright green in colour and had thick filaments. As P concentrations decreased, the chlorophyll a : total P ratio increased (Fig. 10).

The large fluctuations in nutrient concentrations in the south-western bay were due to the more or less poorly functioning treatment plant. The distribution of plant nutrients and algae along a transect from Fundo Bay (F) to station A (Fig. 1) indicated a gradual dilution and algal uptake of nutrients (Fig. 11). The chlorophyll a concentration remained almost the same at all stations, however (Fig. 14). The dissolved nutrients along the transect were available for further algal growth according to laboratory assays with *Microcystis*; the sample collected closest to ETE-Sul excepted (Fig. 12) where instead a population of very small bacteria developed.

A phytoplankton community dominated by green algae enters the bay from Rio Fundo. The total N to total P ratio (TN:TP, by weight) was about 30 in the incoming water (Hilmer 1978), and the green algae were favoured in the inner part of the bay. In the vicinity of the treatment plant the TN:TP ratio dropped, as sewage water TN:TP was <10 (Hilmer 1978), and blue-green

algae started to dominate. Towards the open lake the TN:TP ratio increased again as total P decreased, but the blue-green algae were already established by this point.

From a survey of phosphorus and phytoplankton in Lago Paranoá (Fig. 1) at the five main stations (A-E), in the inner bays outside the tributaries, at the outflow (Lago Paranoá Dam) and close to the discharges of the treatment plants, relations between chlorophyll a, suspended solids, algal fresh weight and phosphorus concentrations were analysed (Table 7). The high chlorophyll a concentration per unit fresh weight in Fundo Bay was explained by a dominance of green algae in the sample. With data from station C, D and Paranoá Dam (Fig. 1), the following relationships for algal fresh weight (FW), suspended solids (SS), chlorophyll a (Chl a) and total P (P) was obtained:

FW	:	SS	:	Chl <u>a</u>	:	P
1,100	:	420	:	3.3	:	1

Phytoplankton Productivity and Respiration

Photosynthetic and respiratory activities were studied on two occasions; once with samples from station A and D and once with samples from stations A and E. Table 8 lists environmental parameters for the samples and chlorophyll a to phosphorus ratios. Temperature and pH showed large variations between the two occasions.

Photosynthetic activity was high at all stations (Table 8) and similar to rates reported in tropical African lakes (Talling 1957, Ganf & Horne 1975, Denny et al. 1978). Verti-

DISCUSSION

The permanent bloom of a monoculture (>99 %) of *Anabaenopsis raciborskii* provides an ideal situation in Lago Paranoá to study algal requirements, growth, and physiological behaviour and adaptation in relation to nutrient concentration and supply.

Variations in water quality within the lake - phosphorus concentrations in particular - did not seem to affect the stability of the *Anabaenopsis* community. Algal filaments varied significantly in shape, however (Cronberg 1978), and phosphorus concentrations in the algae ranged from values typical for cells with sufficient phosphorus to values representative for cells under moderate to extreme P deficiency (Healey 1978, Reynolds 1978).

Algal Response to Nutrient Supply

Phosphorus was already identified as the primary growth-controlling nutrient in Lago Paranoá water in laboratory assays with pure cultures. The *Anabaenopsis* population failed to grow under laboratory conditions, however, when only phosphorus was added. Addition of both nitrogen and phosphorus stimulated *Anabaenopsis*, but after 15 days the algae collapsed. A few attempts to cultivate *Anabaenopsis* were fruitless. *In situ*, *Anabaenopsis* was stimulated by addition of phosphorus alone and showed N_2 -fixing properties as well.

cal profiles of gross primary production (Fig. 13) showed that photosynthetic activity in samples from station A was almost three times that of samples from station D (Table 8). The profiles further showed that the photosynthetic zone was limited to the top 2 m. Transparency at station E where the samples were exposed was 57 and 54 cm on the two occasions. The phytoplankton were usually evenly distributed in the top 4 m (Cronberg 1978).

When the photosynthetic rate was calculated per unit biomass (chlorophyll a), activity was highest for station A (Table 8). The rate per unit phosphorus was similar for all stations, however.

Considering the high respiration rates (Table 8), net production is low or even close to zero. In eutrophic Lake George (Uganda) about 90 % of the gross carbon fixation is reported lost via respiration (Ganf & Horne 1975). The lowest respiration, calculated as % of gross production, occurred in Lago Paranoá at station A on both occasions. Assuming that respiration is the same day and night and that gross production equals respiration at station D (i.e. no net production), there was a net particulate production of 4 and 11 % of gross production at station A on the two occasions during the time of exposure. Net production per day would be lower since photosynthetic rates change with light intensity while respiration remains almost the same during the day. No estimations of daily production were possible because light measurements were not feasible. A 12 month vegetation period will, however, maintain a high biomass.

Ammonia was constantly available in the lake water - a phenomenon also observed in uncontaminated waters like Lago Santa Maria (Table 2). In addition, N_2 fixation could meet nitrogen requirements if phosphorus should be in excess. Thus phosphorus is the element that controls algal standing crop in Lago Paranoá as long as the lake is inhabited by a N_2 -fixing blue-green algal population such as *Anabaenopsis raciborskii*. The rapid uptake of phosphorus by the algae, independent of inorganic nitrogen concentrations, confirms how critical all additions of phosphorus are to the lake.

When 2.5 and 5 % sewage effluent was added to lake water, the difference in algal response was less than expected from the differences in phosphorus concentrations (Table 9). Chlorophyll a concentrations were high (ca. 350 µg/l in series 1), however, and factors other than nutrient supply might have limited growth (Table 9, series 1, bag 6). The *Anabaenopsis* population seemed to react negatively to high nutrient levels, resulting in collapse of the algal community (Fig. 3 and 8). The rapid change in pH accompanying growth might have influenced community stability in the weakly buffered lake water (cf. Talling 1976). Fluctuations in pH are reported to be critical to the maintenance of bloom-forming populations (Shapiro 1973, Reynolds & Walsby 1975). Similar behaviour of the algal community was recorded in the laboratory assays upon large additions of Fundo 2 water (cf. Table 6). Direct observations outside the south treatment plant (ETE-Sul, Fig. 1) have shown that *Microcystis* sometimes dominates the phytoplankton and

that total phosphorus then exceeds 250 $\mu\text{g P/l}$.

In the bag with water from the oxidation pond Guarã (Table 4, series 2, bag 5), small green algae from the Guarã water were probably responsible for the major part of the chlorophyll a peak reached after two days (Fig. 8). These green algae represented 1/3 of the initial chlorophyll a concentration in the bag. The "dilution" of the Guarã water and the high growth rate of the green algae probably co-operated to cause the rapid chlorophyll a increase. The generation time for *Anabaenopsis* was, on the other hand, estimated to ca. 2.5 days. The subsequent rapid decline of the algal community was followed by permanent low algal density (Fig. 8).

The relationship between yield of chlorophyll a and total phosphorus in the bioassays was in many respects comparable to chlorophyll a-phosphorus relations in the open lake water (Fig. 15, cf. Fig. 10).

Nutrient limitation studies by means of laboratory bioassays in African tropical lakes have indicated that nitrogen is the primary growth controlling nutrient in mesotrophic to eutrophic waters (Moss 1969, Viner 1973, Robarts & Southall 1977). These laboratory assays have included the use of both *Selenastrium* and natural communities containing N_2 -fixing algal species. In Lake George (Uganda) N_2 fixation could be measured *in situ* (Ganf & Horne 1975). In the oligotrophic man-made lakes (Robarts & Southall 1977) and in Lake Victoria (Evans 1961) phosphorus was considered the primary element limiting algal standing crop.

Relationships Phosphorus-Chlorophyll-Phytoplankton Standing Crop

In phosphorus-limited tropical lakes in Africa dominated by blue-green algae, ratios of chlorophyll a to phosphorus approach 2:1 (Talling pers. commun.). There is a considerable variation in chlorophyll to phosphorus ratios in temperate lakes (Dillon & Rigler 1974, Nicholls & Dillon 1978). In most cases the ratios reported were below 1:1, however (Nicholls & Dillon op. cit.). In some more nutrient rich British lakes, the vernal chlorophyll a concentration in relation to winter dissolved reactive phosphorus was around 2:1 (Esthwaite Water, Talling pers. commun., Reynolds 1978). Increased phosphorus supply in bioassays performed in the English Lake District did not always give proportionate chlorophyll a increases, as the Chl a : P ratio decreased with increased phosphorus concentrations (cf. Reynolds op. cit.). Similar observations were made in Lago Paranoá when phosphorus concentrations were above ca. 100 µg P/l (cf. Fig. 10 and 15). Considering the amount of phosphorus already bound to the algae in the polluted bays, a 2-to 3-fold increase in chlorophyll and algal fresh weight was necessary to reach the cellular phosphorus content in algae at station C (Table 7). In Lake George the concentration of phosphorus in phytoplankton did not change during the year (Viner 1977).

In Lago Paranoá chlorophyll a varied between 0.3-0.7 % of algal fresh weight (Table 7). The lower values referred to the less polluted parts of the lake and were in good agree-

ment with values found in tropical African lakes (Talling 1966). The higher values in the polluted bays were associated with the higher phosphorus concentrations (Table 7). The same tendency was observed in the *in situ* bioassays (Table 9). The chlorophyll a content per unit biomass in Fundo Bay (Table 7) and in the bag with Guará water (Table 9) was extremely high because the major part of the phytoplankton community consisted of green algae. These algae are supposed to have a higher cellular chlorophyll a content (Nicholls & Dillon 1978). In a survey of the literature on cellular chlorophyll content in algae a range of two orders of magnitude (0.1-9.7 % of fresh weight) was found (Nicholls & Dillon 1978). For diatoms and blue-green algae the chlorophyll a content was reported to be 0.4 % of fresh weight. Higher chlorophyll a content is reported for actively growing algae. High light intensities, on the other hand, reduce the chlorophyll content (Nicholls & Dillon 1978).

The particulate matter (suspended solids) in Lago Paranoá represented about 35 % of algal fresh weight for samples from the open lake (Table 7). Dry weight values for phytoplankton vary between 10 and 30 % of fresh weight (Winberg 1971). The higher values are found in algal cultures (Fitzgerald 1969). However, both algal fresh weight and particulate matter (which is influenced by inorganic material to some extent) per unit phosphorus in Lago Paranoá approached values obtained in cultures of algae (Fitzgerald 1969, Healey 1978).

Phosphorus Loading and N_2 Fixation

The presence of ammonia in the lake water made algal N_2 fixation unnecessary. When an excess of phosphorus was supplied, however, inorganic nitrogen was depleted, heterocyst differentiation started and N_2 was fixed from the atmosphere. This was demonstrated in bioassays when only phosphorus was added. When sewage water was added, a low number of heterocysts also developed. This explained the occasional observations of heterocysts outside the south treatment plant (ETE-Sul) and along the transect in the bay (Cronberg 1978) when phosphorus concentrations were high and the TN:TP ratios were low.

Phosphorus is required for N_2 fixation. Exogenous phosphorus is not necessary, however, since internal stores can be utilized (Stewart & Alexander 1971). The presence of ammonia, on the other hand, inhibits nitrogenase synthesis (cf. Lean et al. 1978). A positive linkage between nitrogen fixation and iron availability has been demonstrated (Elder & Horne 1977, Lean et al. 1978). The reservoirs in the Brasilia region contain large quantities of iron (Table 2).

When phosphorus was added separately and in combination with nitrogen in *in situ* assays, similar amounts of chlorophyll a were produced in the bags after 9 days of growth (Table 9, series 2, bag 1 and 3). Algal fresh weight was lower in the bag with only phosphorus added (Table 9), but the algae were still growing (Fig. 8). Assuming that nitrogen to chlorophyll a ratios were similar in the two bags and that added nitrogen

in bag 3 was assimilated (added N:P=10), about 135 mg N (ca. 0.9 mg N/l) must have been fixed from the atmosphere in bag 1 (only $\text{PO}_4\text{-P}$ supplied) during less than 10 days. If the N_2 fixation rate was constant during the 9 days of algal growth, a daily fixation of 75 mg N/m^2 took place. This rate is comparable to N_2 fixation measured *in situ* in Lake George, Uganda, i.e. $58 \text{ mg N/m}^2\cdot\text{day}$ (Ganf & Horne 1975). Lean et al. (1978) obtained an average fixation rate in August of $57 \text{ mg N/m}^2\cdot\text{day}$ in limnocorrals supplied with phosphorus in the eutrophic Bay of Quinte, Lake Ontario. When N_2 fixation was compared to the phosphorus loading in the corral, a N:P ratio of 7 was obtained. The maximum N_2 fixation rate recorded was $125 \text{ mg N/m}^2\cdot\text{day}$ (Lean et al. 1978).

No measurements of N_2 fixation were made in Lago Paranoá. However, the relationships between phosphorus loading and N_2 fixation above support the estimates of N_2 fixation made from chlorophyll and phosphorus values in the bioassays. Besides, a correlation between nitrogen availability and chlorophyll a content in phytoplankton has been demonstrated (Nicholls 1976). Nitrogen fixation of the *Anabaenopsis* could thus supply the entire requirement of nitrogen for the phosphorus loading of $7 \text{ mg P/m}^2\cdot\text{day}$ in the bag. The present estimated loading on the lake is $1.9 \text{ g P/m}^2\cdot\text{year}$, i.e. $5.2 \text{ mg P/m}^2\cdot\text{day}$ (Hilmer 1978).

CONCLUSIONS

The monitoring part of the investigation clearly revealed the variations in water quality within Lago Paranoá due to pollu-

tion sources. Those variations were more pronounced than seasonal changes and their related pollution (stormwater pollution, etc.).

A close linkage between phosphorus and chlorophyll a concentrations was demonstrated. However, variations in plant nutrients within the lake strongly influenced the phosphorus content of the phytoplankton (Table 7). This was shown by high chlorophyll a to phosphorus ratios (3.5:1) at the main station C (Fig. 1). Lower and fluctuating ratios (Fig. 10) were found closer to the southwestern bay (station A), resulting from the continuous nutrient contributions from a treatment plant and the polluted tributary Rio Fundo.

Primary productivity and respiration measurements indicated that net production occurs in the polluted southwestern bay where phosphorus concentrations are favourable for growth. The data show that the southwestern bay can be compared to a large continuous culture with a small but constant net production and algal cells containing sufficient phosphorus. The open lake, on the other hand, resembles a huge batch culture in a stationary growth phase with a balance between algal growth and decay. The open lake constantly receives contributions of cells with sufficient phosphorus, however, and biomass may gradually increase.

Close to the south treatment plant the water contains large amounts of dissolved nutrients available for algal growth. Green algae enter the lake continuously via Rio Fundo. However,

outside the south treatment plant there is a sudden shift in algal species dominance to blue-green algae. This can be explained by unfavourable nitrogen to phosphorus ratios in the water, grazing and/or toxic substances.

The bioassays clearly confirmed that phosphorus is the main element controlling phytoplankton growth in Lago Paranoá. The algal community in the polluted part of the lake was able to increase its biomass solely by reducing the cellular phosphorus to about 0.1 % of algal fresh weight as observed at station A and in the bioassays.

If the naturally present inorganic nitrogen concentration in the lake is depleted (contrary to expectations), heterocysts develop on the algal trichomes and nitrogen is fixed from the atmosphere. This was shown when phosphorus alone was added in bioassays *in situ*. Nitrogen was fixed to meet the entire nitrogen requirement of the algae. A rapid depletion of inorganic nitrogen compounds from the water did not affect the phosphorus uptake rate. Fixation of N_2 alone was sufficient to produce the algal biomass achieved at a phosphorus loading of $7 \text{ mg P/m}^2 \cdot \text{day}$. This is about 1.5 times the present loading on the lake (Hilmer 1978).

Thus, an elimination of nitrogen to combat eutrophication is not feasible and phosphorus removal is the only way to retard algal growth. Therefore, the point sources as well as other sources of phosphorus-contaminated water must be brought under control. Since water renewal is rapid (1 year) in this

large lake and no significant organic sediments have been deposited (Björk 1975, Enell 1978), a rapid recovery of the lake after sewage diversion and efficient reduction of phosphorus loading can be predicted, and the recreational value of the lake can be regained.

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Table 1. Morphometric and hydrological data for Lago Paranoá, Brasília.

Surface area	40 km ²	Altitude M.a.s.l.	1 000 m
Volume	540 Mm ³	Drainage area	1 011 km ²
Maximum depth	37 m	Water renewal time ca.	1 year
Mean depth	13.5 m		

Table 2. Physical-chemical and biological characteristics of Lago Paranoá and Lago Santa Maria. Average values at 1 m depth, September 1976 - October 1977¹⁾.

Parameter		L. Paranoá*	L. Santa Maria
Temperature	C°	23.5	23.5
Secchi disc depth	m	0.5	4.5
Conductivity	µS ₂₅ /cm	40	12
pH		8.6	7.2
Alkalinity	meq/l	0.44	0.15
Fe	mg/l	0.3	0.4
Ca	mg/l	4.5	1.3
NH ₄ -N	µg/l	300	500
(NO ₂ +NO ₃)-N	µg/l	75	110
Total N	µg/l	1 500	1 000
PO ₄ -P	µg/l	<5	<3
Total P	µg/l	30	<10
Chlorophyll <u>a</u>	µg/l	105	<10
Phytoplankton fresh weight	mg/l	27	0.4

* Station C (Fig. 1).

¹⁾ Data from Cronberg (1978), Enell (1978) and Lindmark (1978).

Table 3. Experimental design for laboratory bioassays.

Flask no.		Composition		Test organism	
Series 1	1	Lago Paranoá	control	<i>Microcystis</i>	
	2	"	+ N	"	
	3	"	+ P	"	
	4	"	+ N,P	"	
	5	"	+ N,P	<i>Scenedesmus</i>	
	6	"	control	natural algal community	
	7	"	+ N	"	"
	8	"	+ P	"	"
	9	"	+ N,P	"	"
Series 2	1	Lago Paranoá	control	natural algal community	
	2	"	+ 10 % Fundo headwaters	"	"
	3	"	+ 25 % "	"	"
	4	"	+ 50 % "	"	"
	5	"	+ 10 % "	"	"
	6	"	+ 10 % "	"	"
	7	"	+ 5 % Fundo 2	"	"
	8	"	+ 10 % "	"	"
	9	"	+ 25 % "	"	"
	10	"	+ 10 % "	<i>Microcystis</i>	
	11	"	+ 10 % "	<i>Scenedesmus</i>	

Table 4. Experimental design, total P and PO₄-P for *in situ* bioassays. Series 1 and 2 were performed in 145 l bags and series 3 in 1400 l bags.

	Bag no.	Composition	Total P µg/l	PO ₄ -P µg/l
Series 1	1	Lago Paranoá		
		control	35	<3
	2	"		
		+ 8 fishes, <i>Tilapia nilotica</i>	35	<3
	3	"		
		+ 25 % Fundo headwaters	27	<3
	4	"		
		+ 50 % Fundo headwaters	18	<3
Series 2	1	Lago Paranoá		
		+ 2.5 % sewage effluent from ETE-Sul	140	82
	6	"		
		+ 5 % sewage effluent from ETE-Sul	245	165
	7	"		
		+ 5 % Fundo 2	110	75
	8	"		
		+ 5 % sewage effluent from ETE-Sul after treatment with Al ₂ (SO ₄) ₃ for P-removal	35	<3
Series 2	1	Lago Paranoá		
		+ P	137	100
	2	"		
		+ N	37	<3
	3	"		
		+ N, P	137	100
	4	"		
		+ 2.5 % sewage effluent from ETE-Sul	115	63
Series 3	1	Lago Paranoá		
		+ 5 % sewage effluent from ETE-Sul	200	127
	6	"		
		+ 5 % effluent from Guará oxidation pond	180	76
	7	"		
		control	37	<3
	8	"		
		control	37	<3
Series 3	1	Lago Paranoá		
		control	56	<3
	2	"		
		+ 2.5 % sewage effluent from ETE-Sul	69	13
Series 3	3	"		
		+ 2.5 % effluent from Guará oxidation pond	178	87
Series 3	4	"		
		+ 2.5 % effluent from Guará oxidation pond	178	87

Table 5. Chlorophyll a in laboratory assays on nutrient-enriched water from Lago Paranoá after 12 days of incubation (series 1).

Test organism	Chlorophyll <u>a</u> , µg/l			
	control	+ N	+ P	+ N,P
<i>Microcystis</i>	11	12	74	178
<i>Scenedesmus</i>	----- no assay -----			94
Natural algal community	--- algae collapsed ---			280 ⁺

⁺ initial lake chlorophyll a (60 µg/l) subtracted

Table 6. Phytoplankton biomass (calculated as percent of initial biomass) in laboratory assays after 6 days of incubation (series 2).

Composition	Biomass, % of initial
Lago Paranoá + 10 % Fundo headwaters	30
" " + 25 % " "	30
" " + 50 % " "	20
" " + 10 % " " + P	20
" " + 10 % " " + N,P	180
" " + 5 % Fundo 2	180
" " + 10 % "	110
" " + 25 % "	90

Table 7. Data from a survey of phosphorus, chlorophyll a, phytoplankton fresh weight and suspended solids in Lago Paranoá, October 26, 1977. Sampling stations in Fig. 1.

Station	Total P	Diss. P	Chl <u>a</u>	FW	SS	Chl <u>a</u> /	% Chl <u>a</u> /	% TP/	% PP/	% Chl <u>a</u> /
	-----	µg/l	-----	--mg/l--	TP	FW	FW	FW	FW	SS
A	242	43	246	50	23	1.0	0.49	0.48	0.40	1.1
B	76	23	161	48	14.2	2.1	0.33	0.16	0.11	1.2
C	34	<5	139	39	13.4	4.1	0.36	0.09		1.0
D	34	<5	103	37	16.6	3.0	0.28	0.09		1.1
E	70	<5	142	33	18.8	2.0	0.43	0.21		0.76
Fundo Bay	233	105	208	29	34.4 ⁺	0.89	0.72	0.80	0.44	0.76
ETE Sul	387	177	308	52	29.2	0.80	0.60	0.74	0.40	1.1
Gama Bay	94	15	133	29	32.9 ⁺	1.4	0.46	0.32	0.27	0.40
Paranoá Dam	38	<5	128	39	14.8	3.4	0.33	0.10		0.86
Torto Bay	25	<5	57	32	19.8 ⁺	2.3	0.18	0.08		0.29
ETE Norte	224	23	118	30	19.7	0.53	0.39	0.75	0.66	0.60
Bananal Bay	58	<5	90	32	21.9 ⁺	1.6	0.28	0.18		0.41

FW= phytoplankton fresh weight

SS= suspended solids; ⁺observed soil interferences

TP= total P, PP= particulate P, Diss.P= total dissolved P

Table 8. Physical-chemical characteristics, gross photosynthesis and respiration for water samples from stations A, D and E in Lago Paranoá.

Parameter	Sampling station			
	A ^{a)}	D ^{a)}	A ^{b)}	E ^{b)}
Temperature	24.4	24.4	26.7	26.7
Transparency	47	70	40	54
pH	7.75	7.35	8.25	8.35
NH ₄ -N	n.a.	n.a.	86	381
Total P	99	37	117	60
Chlorophyll <u>a</u>	126	83	128	87
Chlorophyll <u>a</u> / Total P (by weight)	1.3	2.2	1.1	1.5
Maximum gross photosynthesis per unit volume	1.49	0.52	1.17	0.76
	g O ₂ /m ³ ·h			
Maximum gross photosynthesis per unit biomass	11	6.3	9.1	8.7
	mg O ₂ /mg Chl <u>a</u> ·h			
Community respiration	0.21	0.08	0.16	0.13
	g O ₂ /m ³ ·h			
Gross photosynthesis per unit area	2.0	0.73	1.65	0.83
	g O ₂ /m ² ·h			

a) integrated sample 0-0.5 m, 1977-12-02

b) " " 0.2-2 m, 1977-12-09

n.a.=no analysis

Table 9. Total phosphorus, chlorophyll a and phytoplankton fresh weight concentrations and relationships in *in situ* bag experiments after 11 days (series 1) and 9 days (series 2).

Bag no. Composition		Total P µg/l	Chl <u>a</u> µg/l	FW mg/l	Chl <u>a</u> / TP	% Chl <u>a</u> / FW	% TP/ FW
Series 1	Lake						
	1	initial	35	113	27	3.2	0.13
		control	32 ⁺	118 ⁺	40 ⁺	3.7	0.08
	2	+ fish	46	146	70	3.2	0.07
	4	+ 50 % Fundo headwaters	16 ⁺	43 ⁺	25 ⁺	2.7	0.06
	5	+ 2.5 % sewage	135	359	83	2.7	0.16
	6	+ 5 % sewage	235	342	86	1.5	0.27
	7	+ 5 % Fundo 2	108	304	78	2.8	0.14
Series 2	8	+ 5 % sewage-P	32	150	69	4.7	0.05
	Lake						
	1	initial	37	65	22	1.8	0.17
		+ P	108	207	60	1.9	0.18
	2	+ N	34	96	53	2.8	0.06
	3	+ N, P	126	221	84	1.8	0.15
	4	+ 2.5 % sewage	101	218	53	2.2	0.19
	5	+ 5 % sewage	174	262	62	1.5	0.28
	6	+ 5 % Guará	129 ⁺⁺	341 ⁺⁺	30 ⁺⁺	2.6	0.43
	7	control	33	78	40	2.4	0.08
	8	control	30	72	34	2.5	0.09

FW= phytoplankton fresh weight
 TP= total phosphorus
 += data after 8 days; ++ = data after 5 days

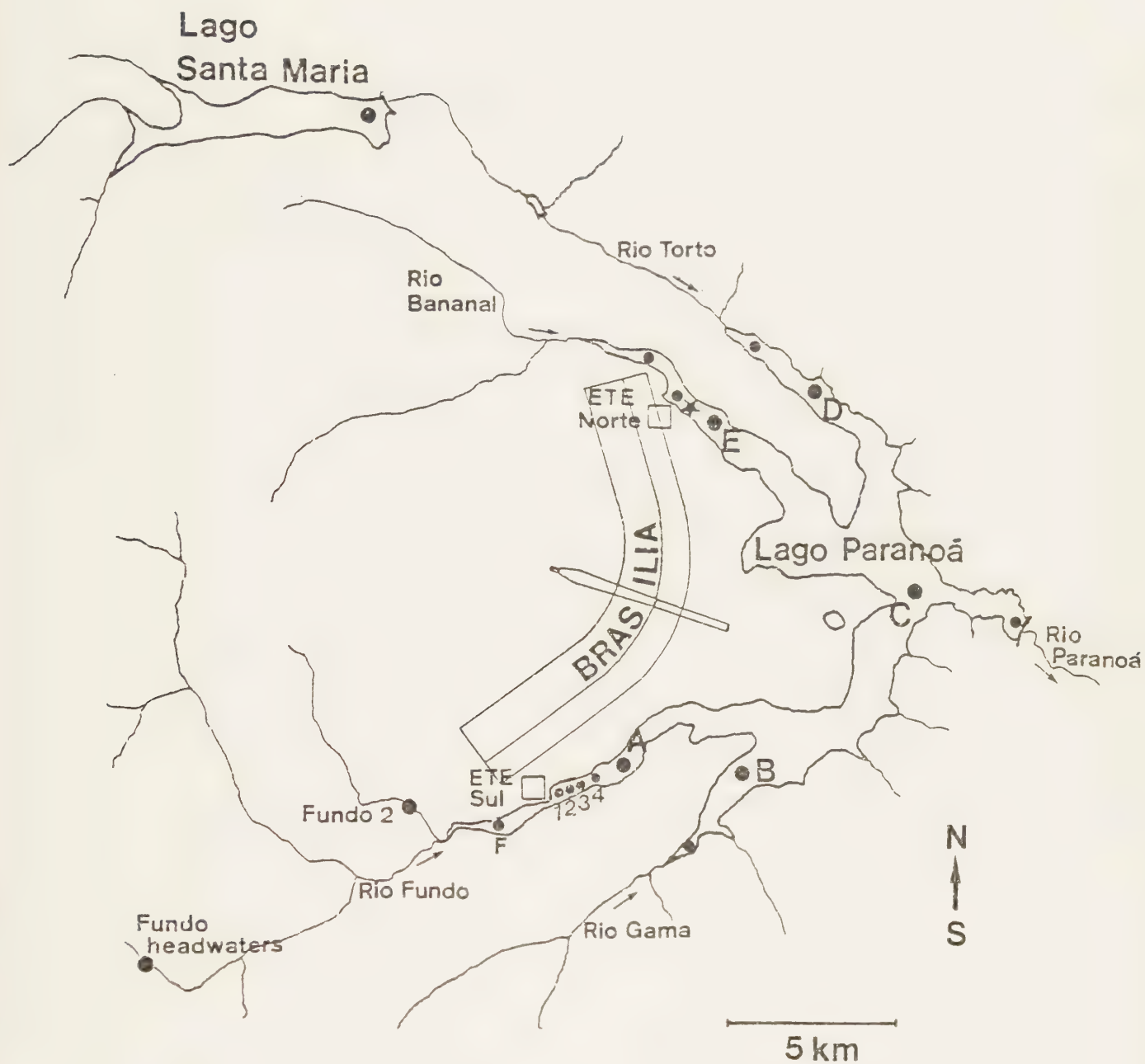


Fig. 1. Lago Paranoá and Lago Santa Maria. Sampling stations (●) and site for *in situ* bioassays (★).

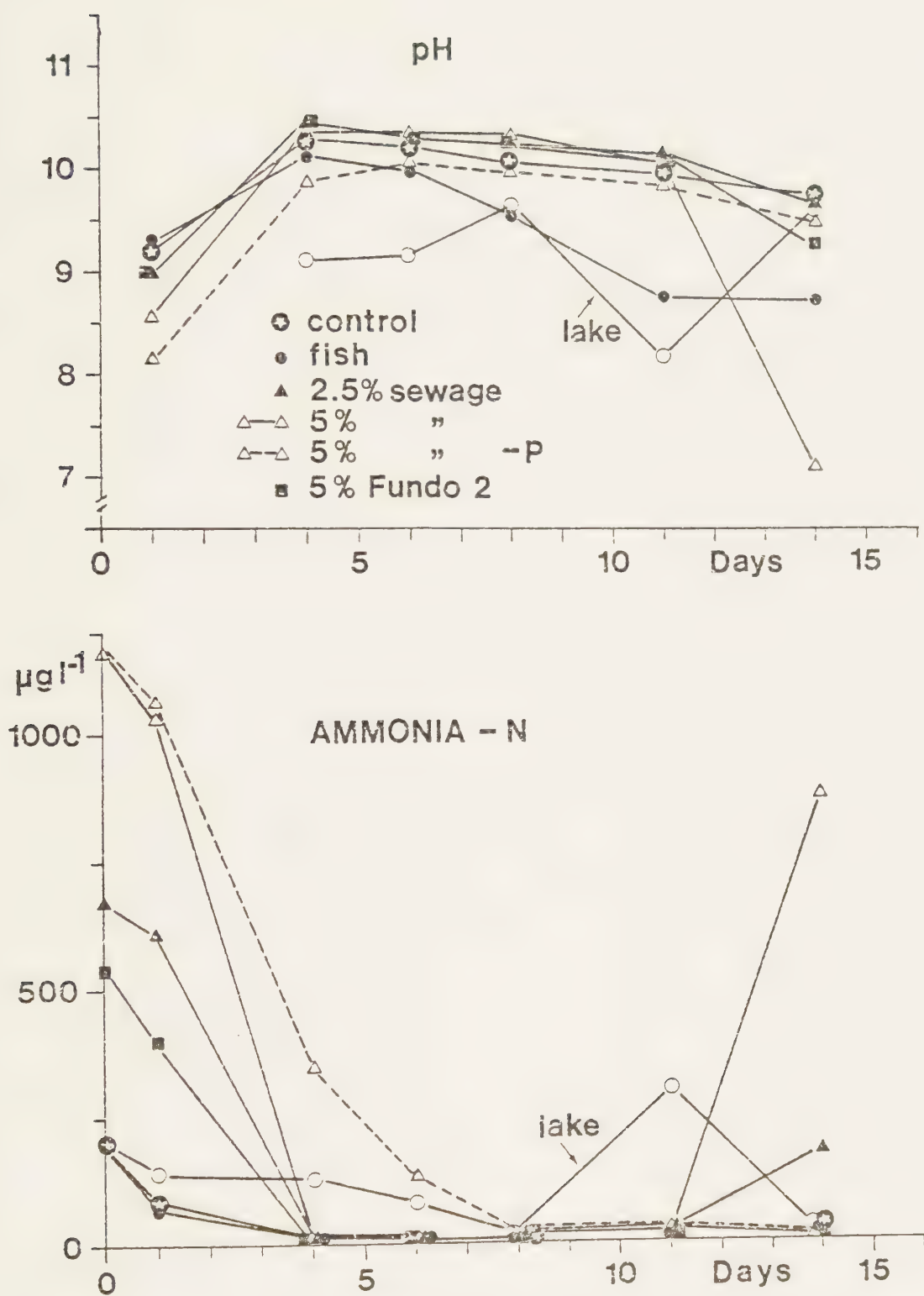


Fig. 2. pH and residual ammonia after addition of polluted water (sewage and Fundo 2) and fish to Lago Paranoá lake water in *in situ* bioassays, October, 1976 (series 1).

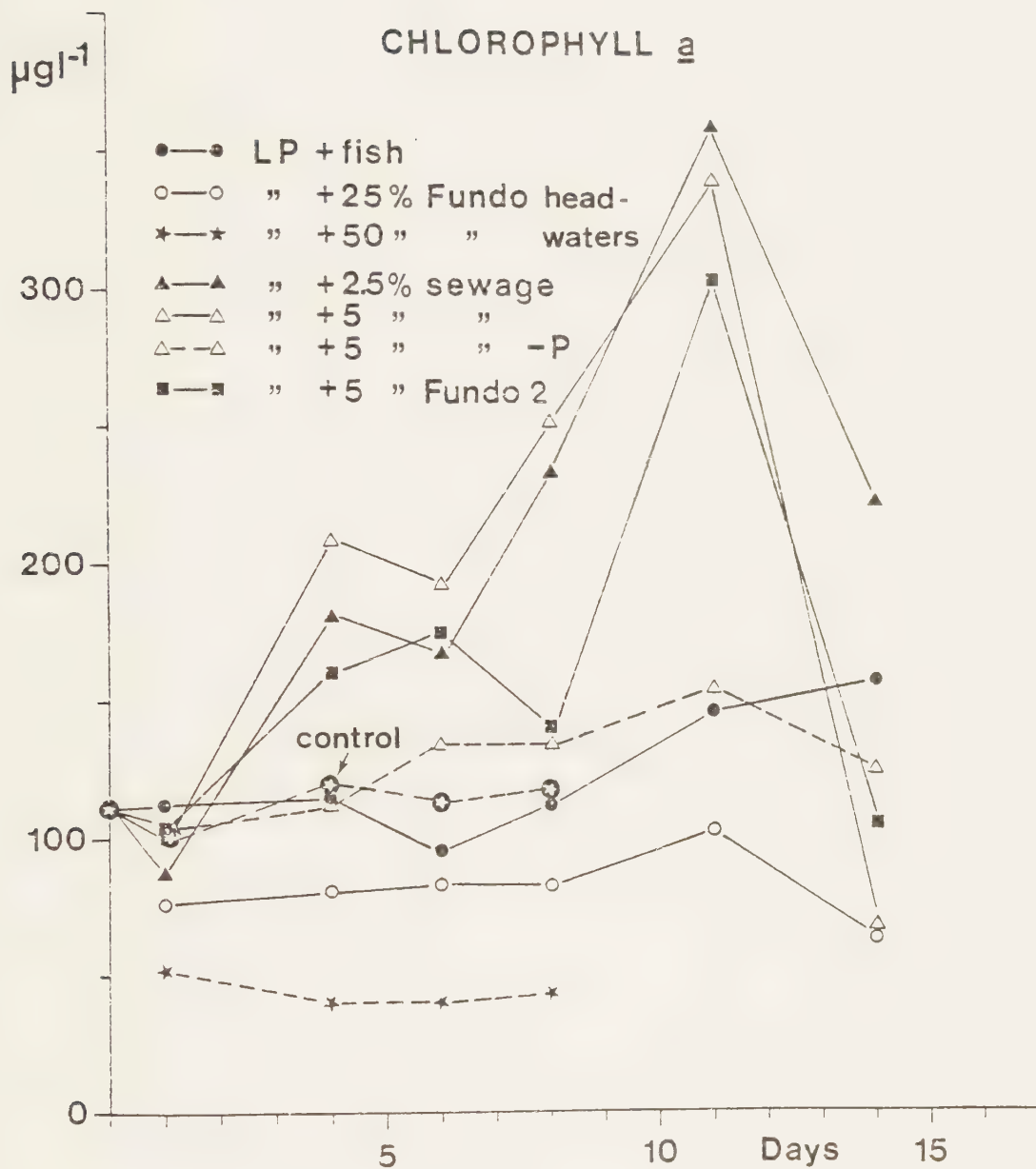


Fig. 3. Chlorophyll a concentrations after additions of polluted water, uncontaminated water (Fundo headwaters) and fish to Lago Paranoá lake water in *in situ* bioassays, October, 1976 (series 1).

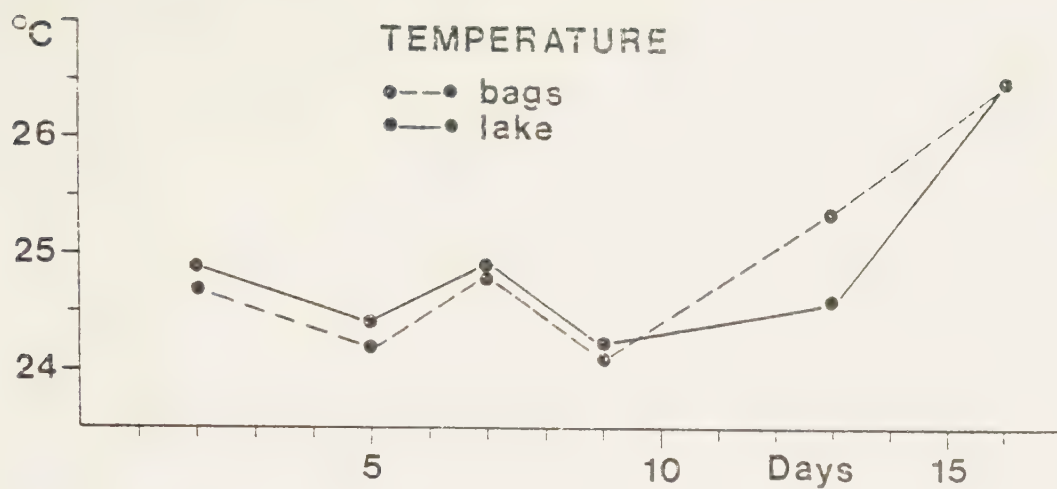


Fig. 4. Temperature in lake water and bags during *in situ* bioassays, November, 1977 (series 2).

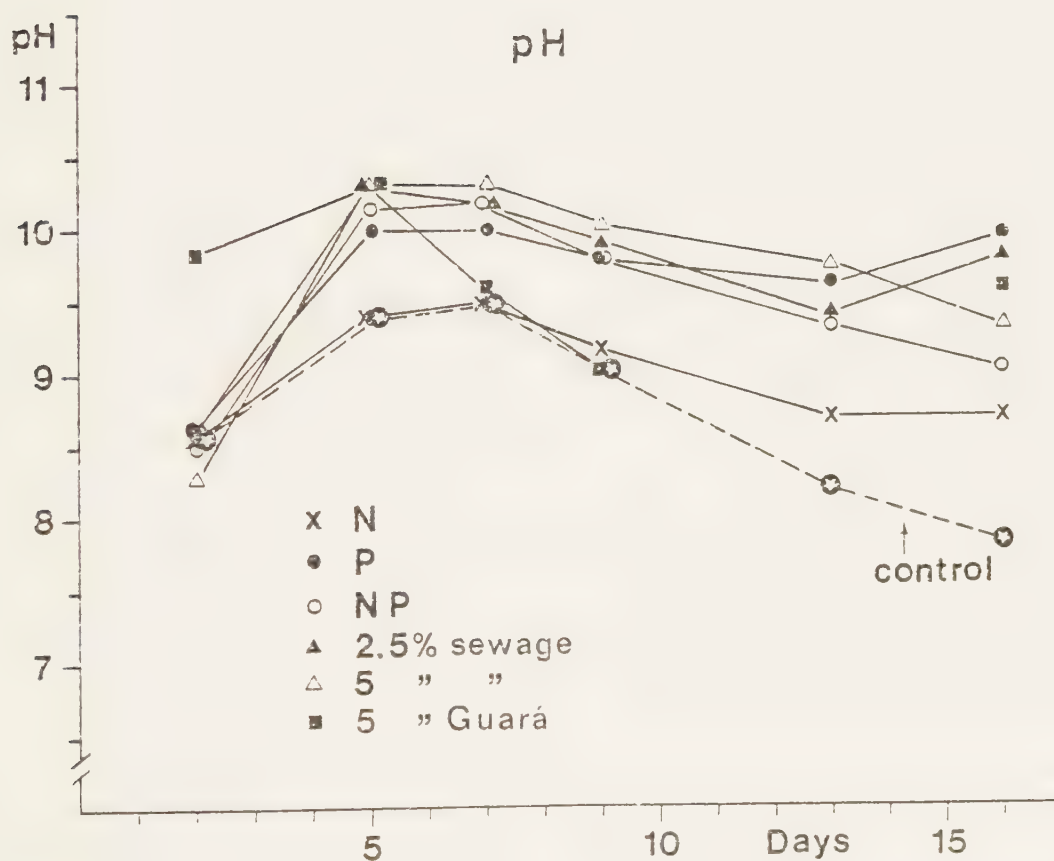


Fig. 5. pH after nutrient and sewage water additions to Lago Parano lake water in *in situ* bioassays, November, 1977 (series 2).

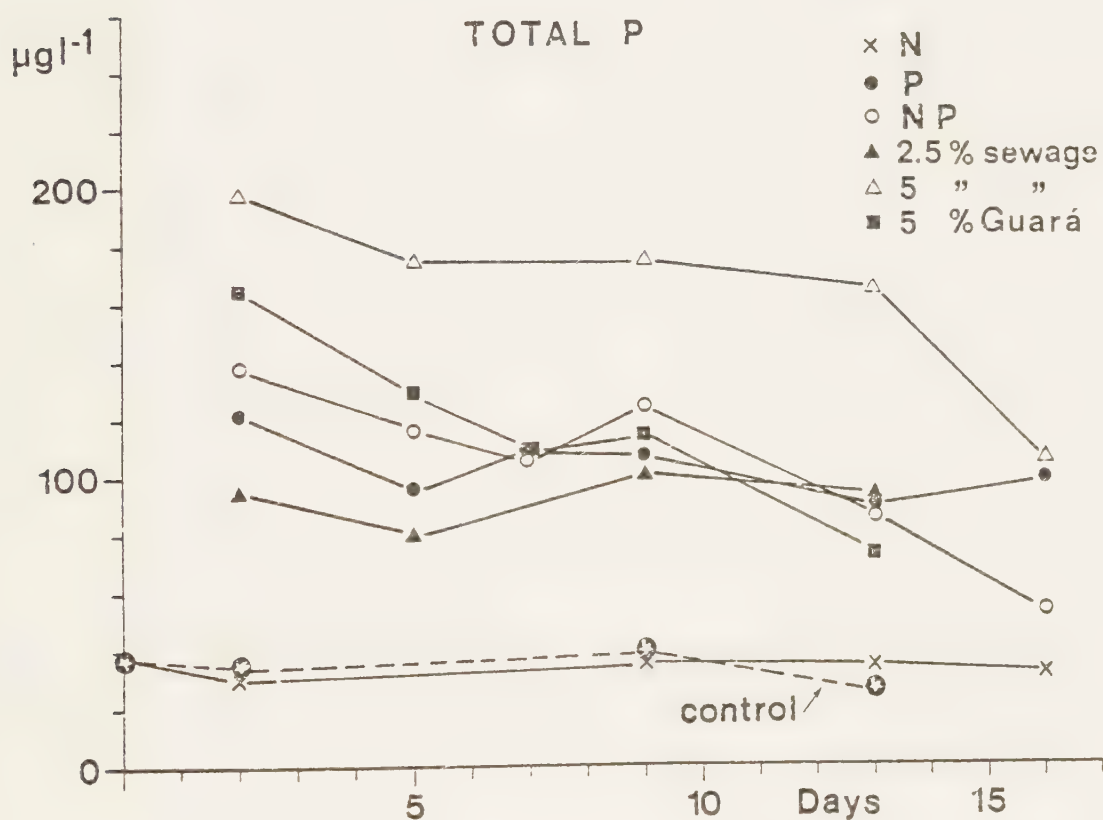
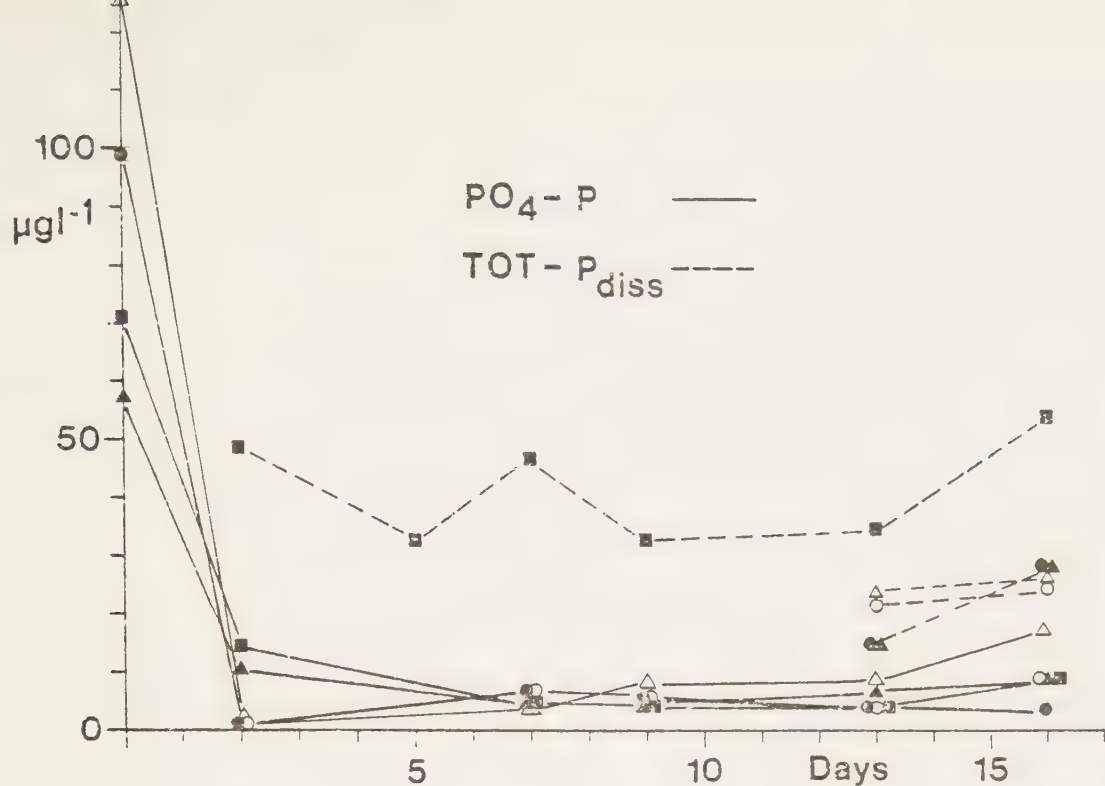


Fig. 6. Dissolved and total phosphorus after nutrient and sewage water additions to Lago Parano lake water in *in situ* bioassays, November, 1977 (series 2).

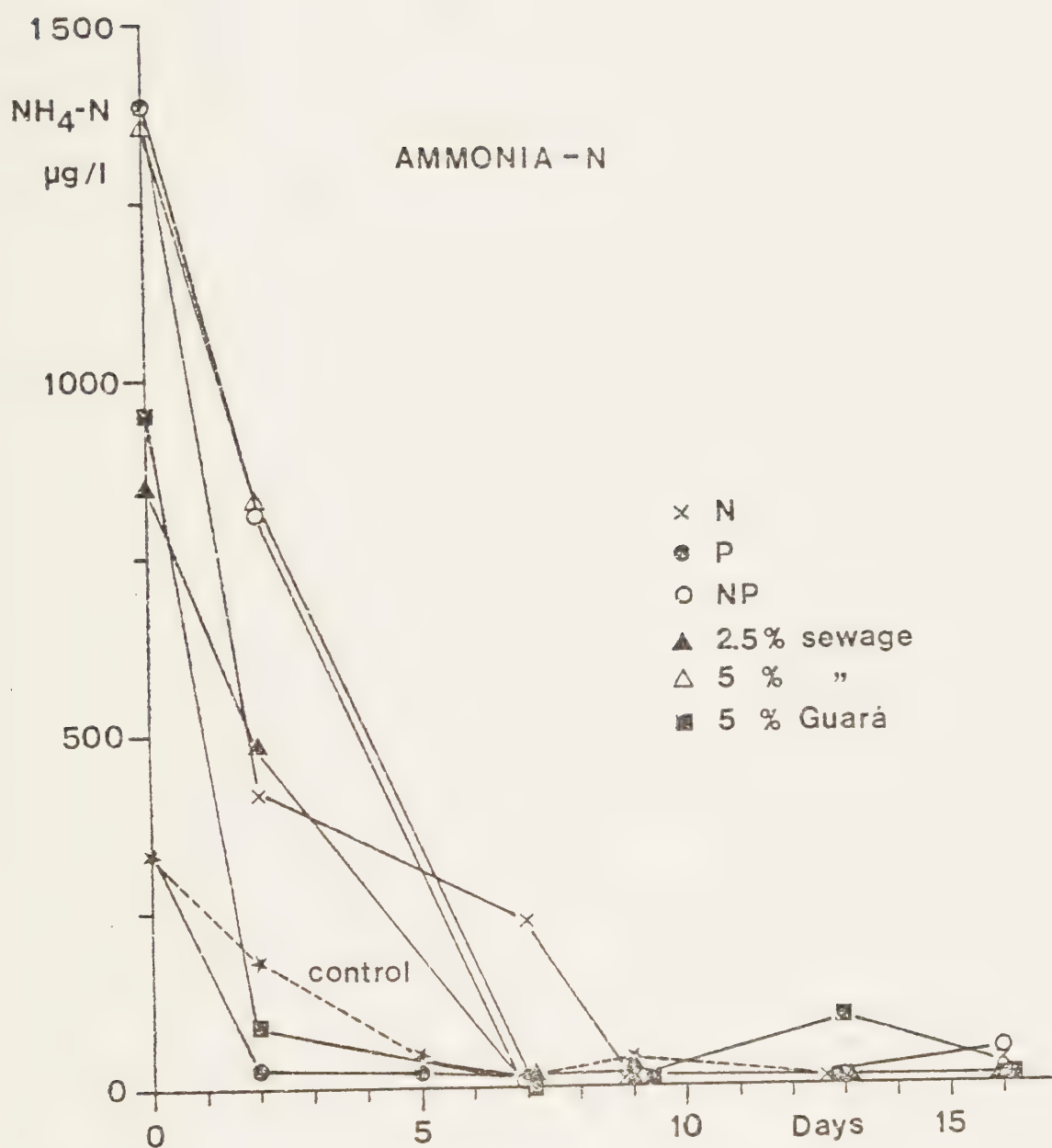


Fig. 7. Residual ammonia after nutrient and sewage water additions to Lago Paranoá lake water in *in situ* bioassays, November, 1977 (series 2).

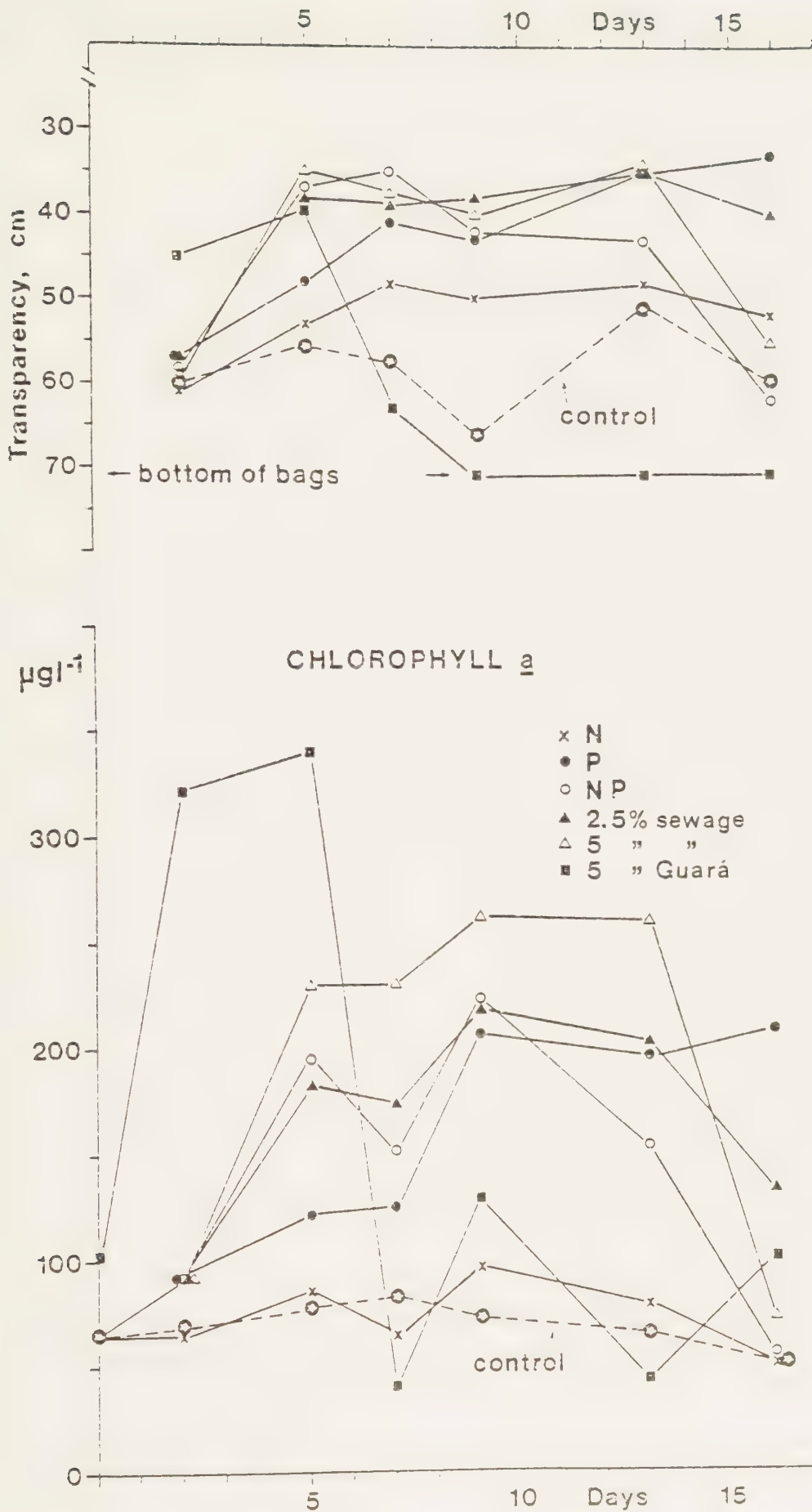


Fig. 8. Transparency and chlorophyll a concentrations in *in situ* bioassays after additions of nutrients and sewage water to Lago Parano water, November, 1977 (series 2).

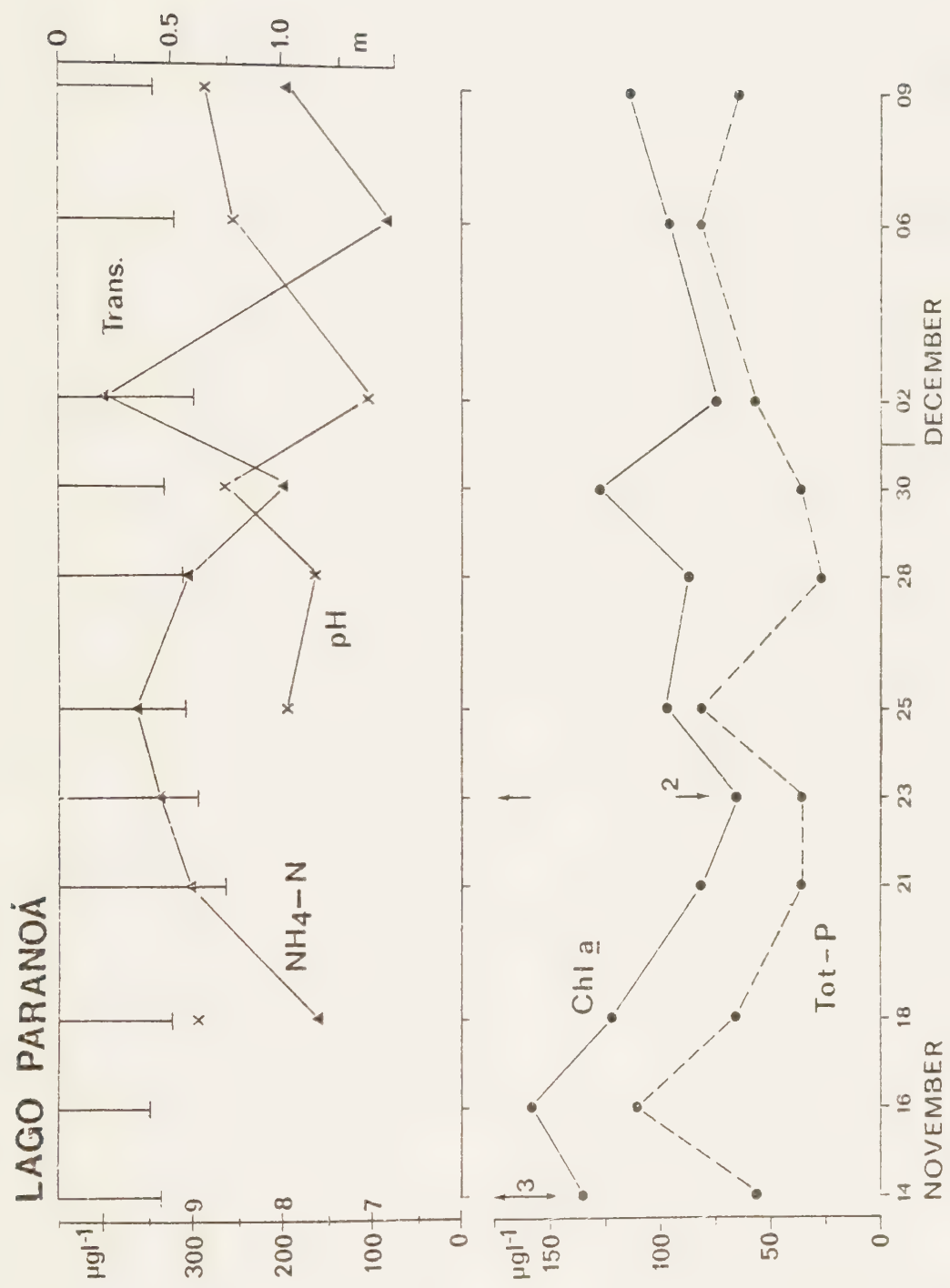


Fig. 9. Transparency and pH, and ammonia, total phosphorus and chlorophyll a concentrations in the northwestern bay of Lago Paranoá (★ Fig.1) during November 14 - December 9, 1977. Arrows indicate the start of *in situ* bioassays (series 3 and series 2).

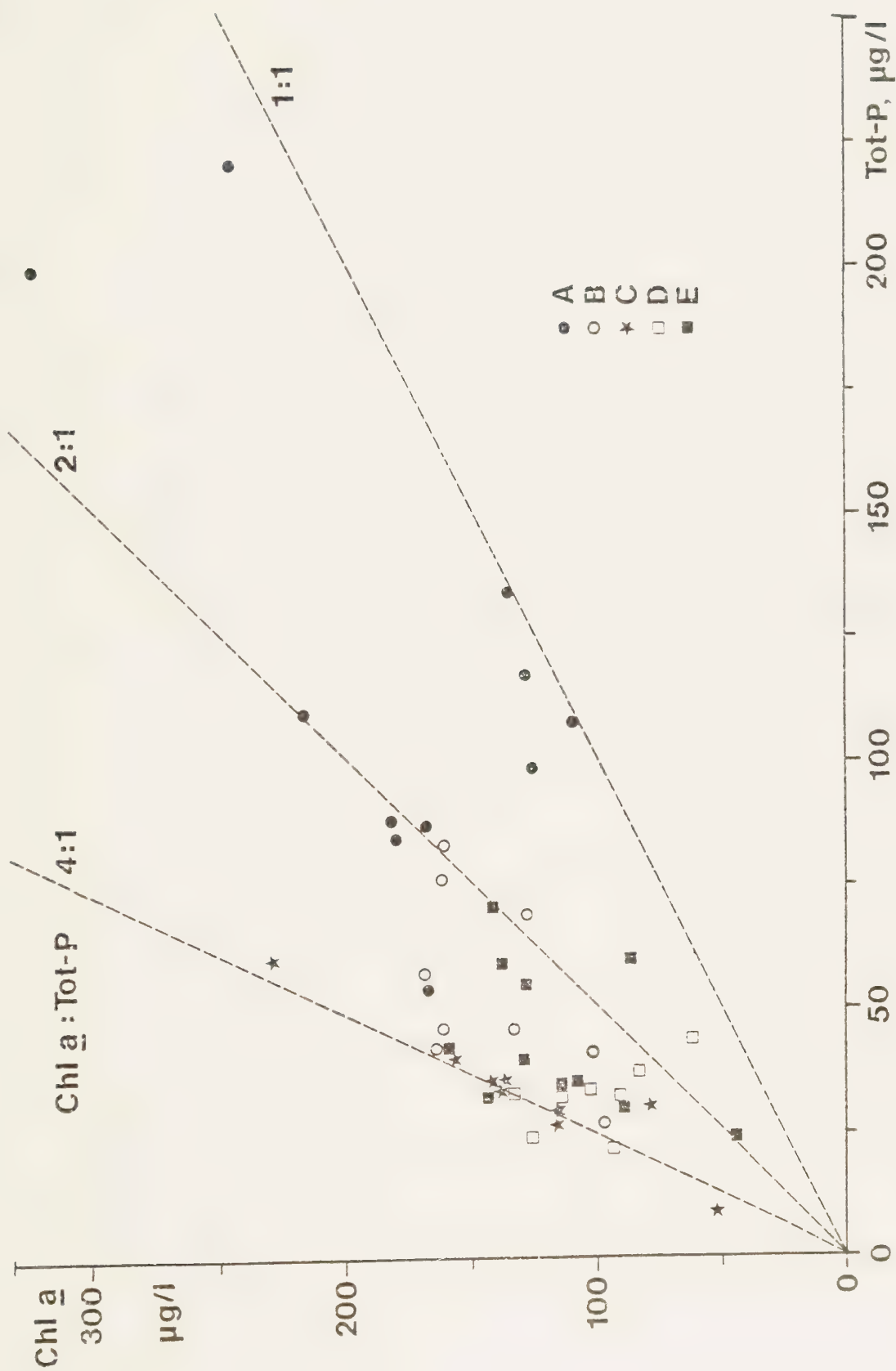


Fig. 10. Relationship between total phosphorus and chlorophyll $\bar{a}$ in samples from different stations (A -E, Fig.1) in Lago Paranoá. Dashed lines represent chl $\bar{a}$: total P ratios 4:1, 2:1 and 1:1, respectively. (Data from September, 1976 - January, 1977 and Oct.-Nov., 1977.)

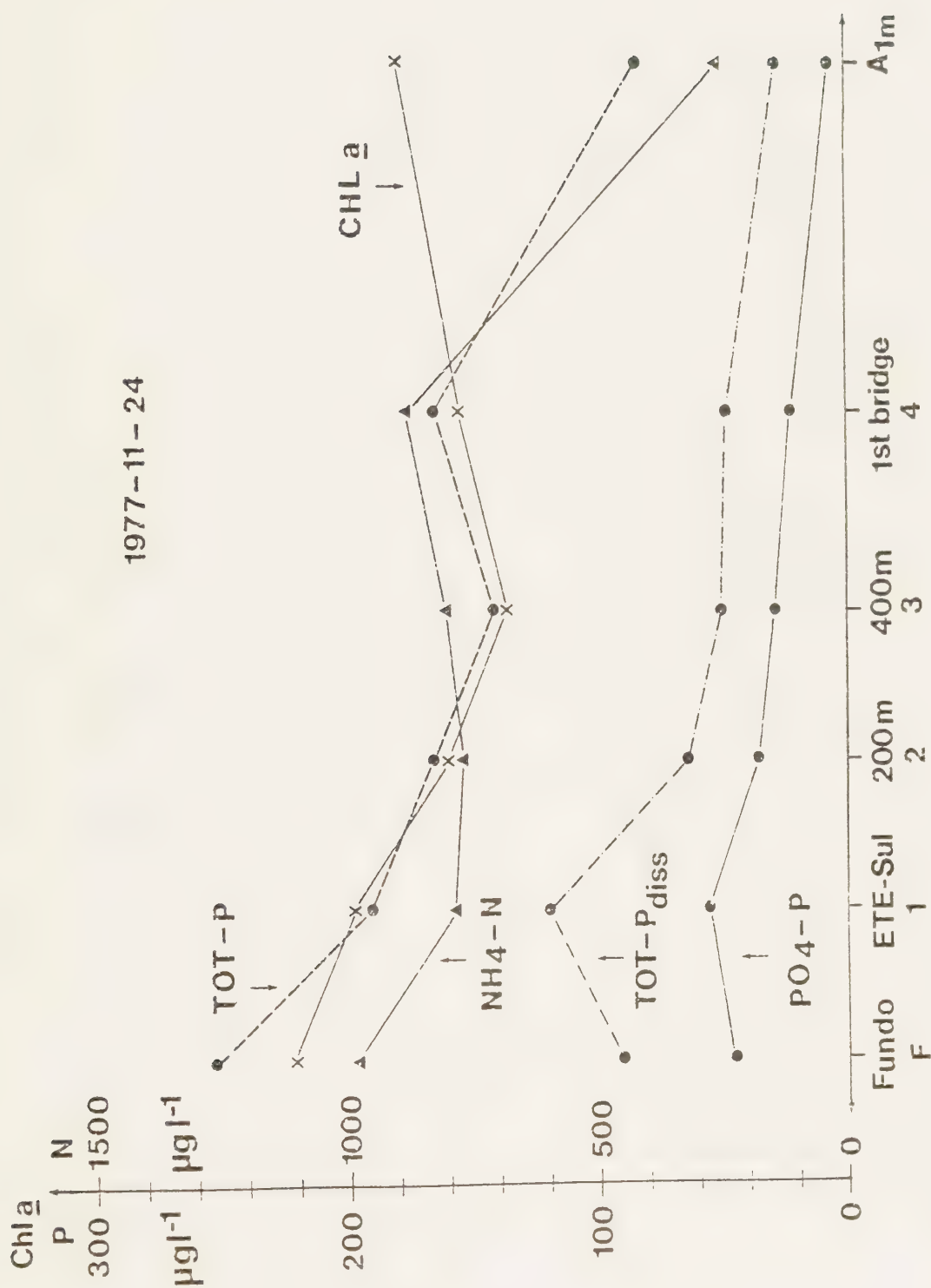


Fig. 11. Phosphorus, ammonia and chlorophyll $\bar{a}$ along a transect from inner Fundo Bay (F) to station A in Lago Paranoá (Fig. 1), November 24, 1977.

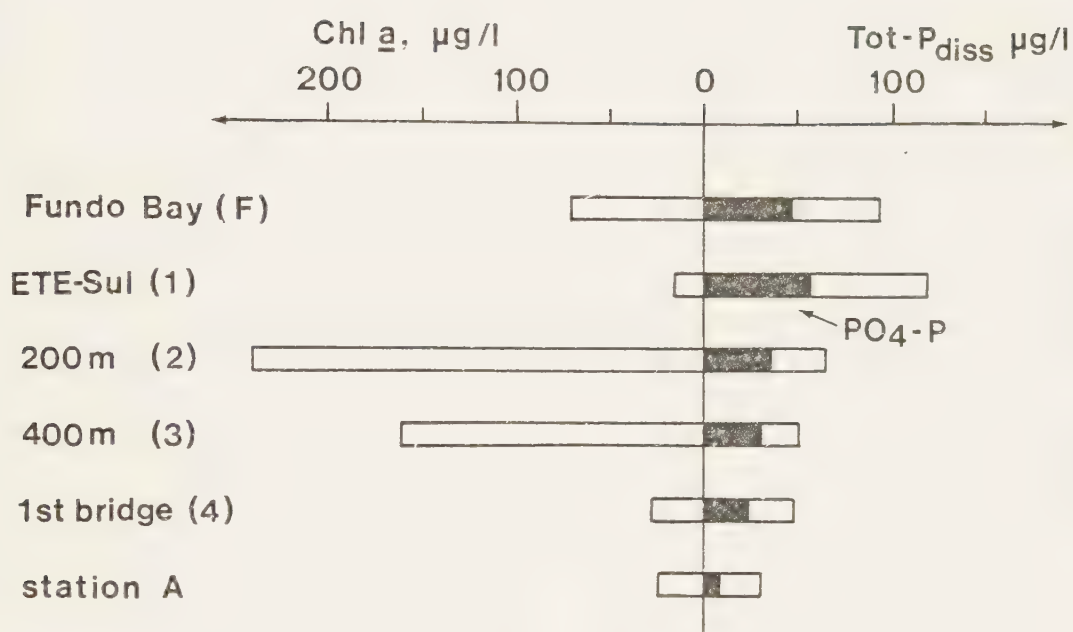


Fig. 12. Phosphorus concentrations (right) and the response of *Microcystis aeruginosa* as chlorophyll *a* in laboratory bioassays (left) in filtered lake water from a transect sampling from inner Fundo Bay to station A in Lago Paranoá, November 24, 1977.

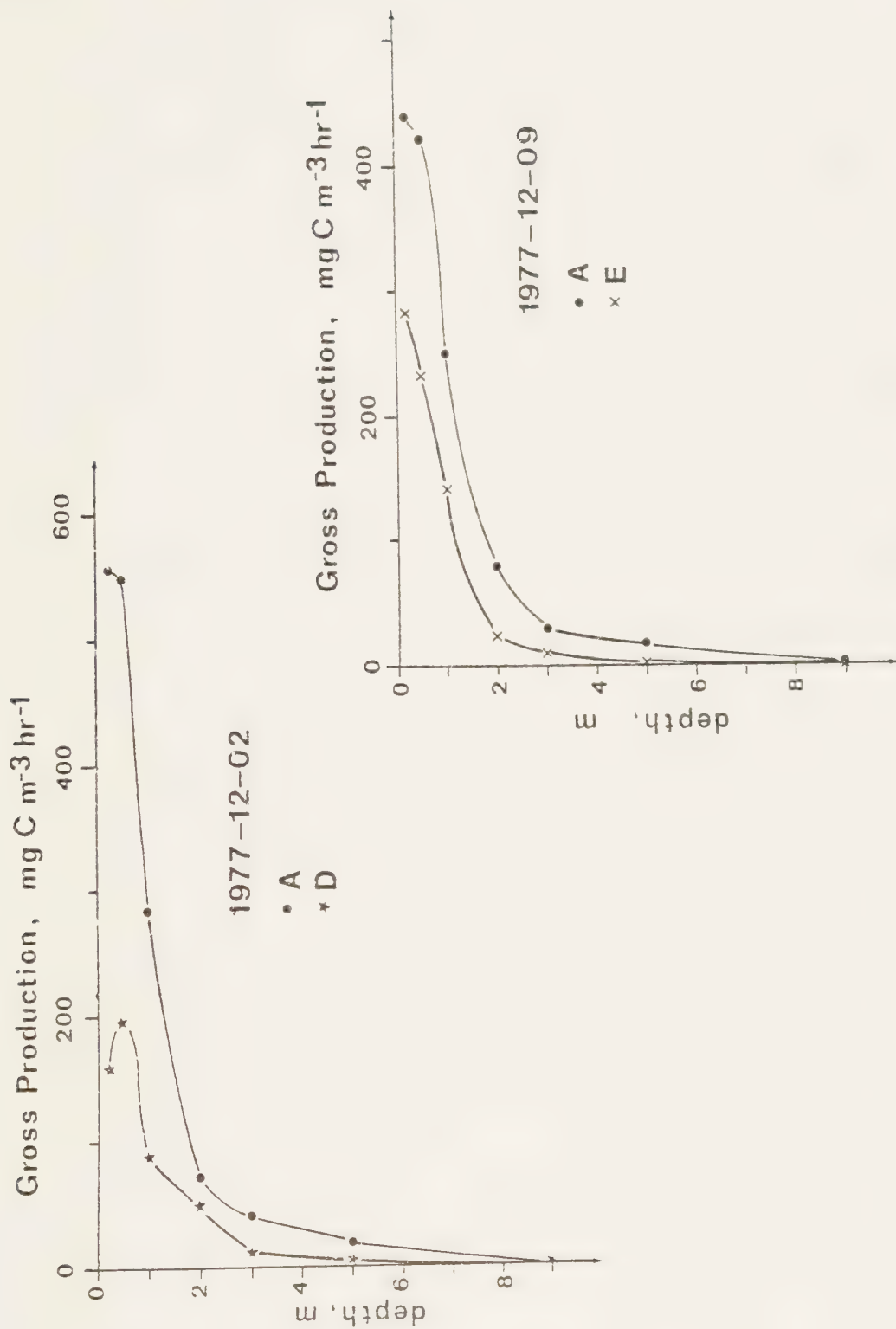


Fig. 13. Vertical profiles of gross primary production for water samples from stations A, D and E exposed at station E in Lago Paranoá. Integrated samples from 0-0.5 m water depths were exposed 1977-12-02 and from 0.2-2 m on 1977-12-09. Oxygen production is converted to carbon assimilation by multiplication with 0.375 (Vollenweider 1971).

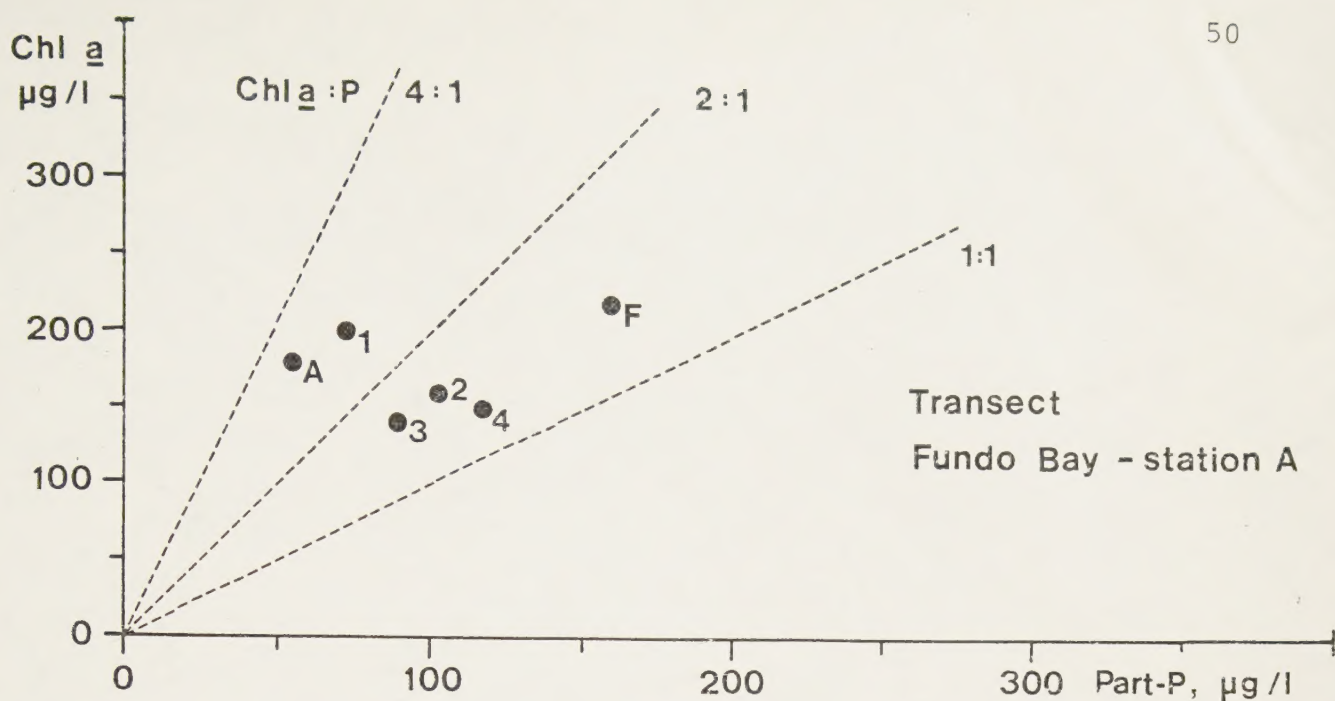


Fig. 14. Relationship between particulate phosphorus and chlorophyll a at stations along a transect from Fundo Bay (F) to station A in Lago Parancá (Fig.1), November 24, 1977. Dashed lines represent chl a : P ratios 4:1, 2:1 and 1:1, respectively.

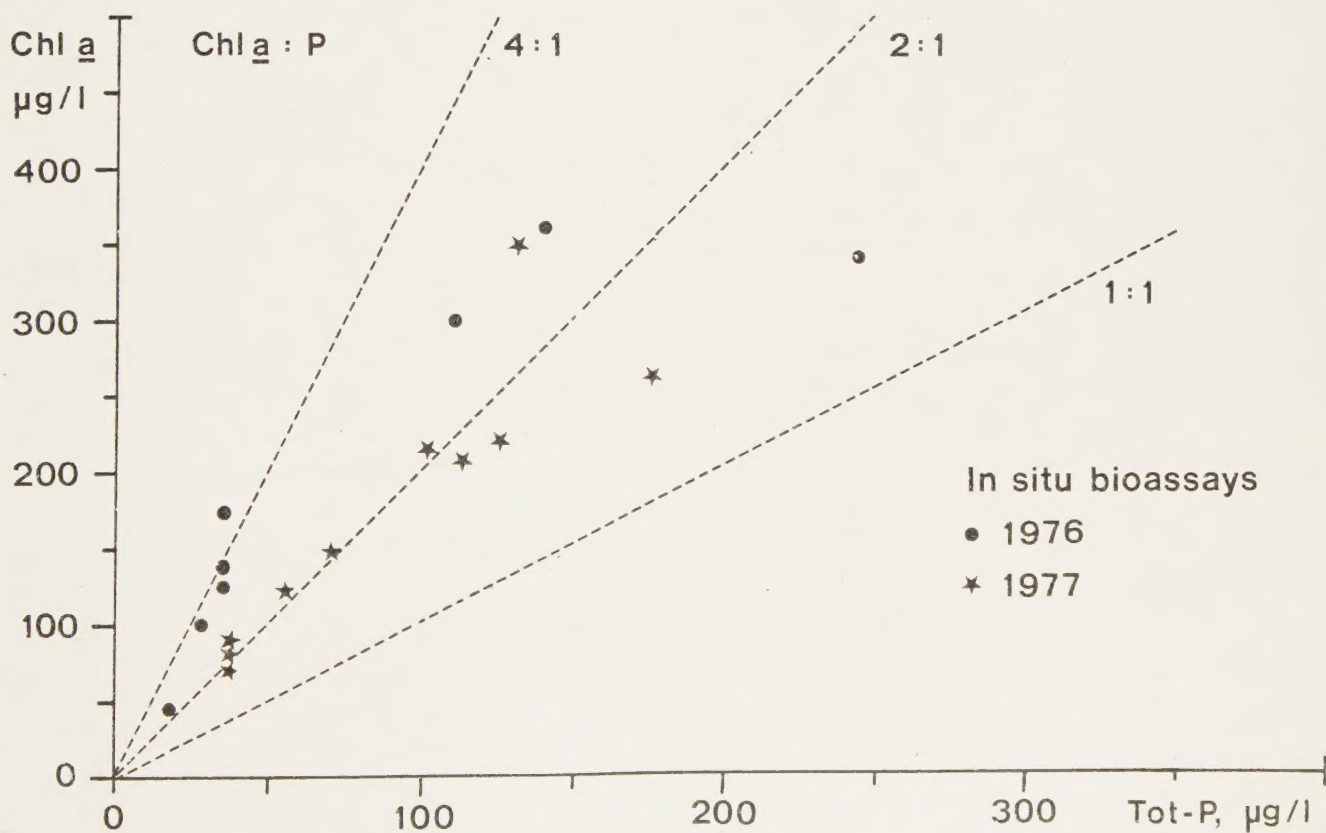


Fig. 15. Relationship between total phosphorus and chlorophyll a in *in situ* bioassays 1976 (series 1) and 1977 (series 2). Dashed lines represent chl a : P ratios 4:1, 2:1 and 1:1, respectively.

